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HYDRATION CAPACITY OF GLUTEN FROM "STRONG" AND "WEAK" FLOURS[1]

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INTRODUCTION

It is a well-known economic fact that there is a great variation in the baking quality of flours prepared from different wheats (*Triticum* spp.). The hard spring wheats, especially those of the northern portion of the Great Plains area, produce a flour which has superior baking qualities, while the softer wheats produce flour of inferior baking qualities. In order to differentiate between these qualities of the flour, the terms "strong" and "weak" flour have been generally accepted. For the purpose of this paper the definition (*8*)[3] adopted by a committee of the National Association of British and Irish Millers will be accepted—

A strong wheat is one which yields flour capable of making large, well-piled loaves; the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily.

Jago (*9, p. 291*) similarly defines "strength" as—

the measure of the capacity of the flour for producing a bold, large-volumed, well-risen loaf.

Obviously those flours not meeting the above conditions must be classed as "weak."

HISTORICAL REVIEW

It is far beyond the scope of the present paper to enter into a complete historical discussion of the work which has been undertaken in attempts to ascertain what factors are responsible for the strength of flour. An enormous amount of literature has accumulated within the last 20 or 30 years, and this literature has been reviewed from time to time by workers

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[3] Reference is made by number (italic) to "Literature cited," p. 417–418.

in this field.[1] Almost every conceivable factor has been investigated with more or less thoroughness; yet—

> no one is believed to have discovered a limiting factor or group of factors which completely solves the problem (3).

Instead we wish to limit the discussion to those papers bearing directly upon our problem, the colloidal properties of the gluten.

It is only within comparatively recent years that the fact has been recognized that the physical state of matter is of paramount importance. When the nature of colloids was first investigated it was supposed that relatively few substances could form colloidal solutions or gels, but it now seems probable that under suitable conditions any substance may be obtained in colloidal form, and it appears almost equally probable that at some future date we may be able to obtain in crystalloidal form those substances which we now know only as colloids. When a substance passes from the crystalloidal to the colloidal state, the physical properties are so altered as to bear almost no resemblance to the original substance; and even when in the colloidal state, the properties are not constant but vary widely, depending upon the size of the colloidal particles, upon their electrical charges, and upon the presence or absence of foreign materials in the dispersion medium. This being the case, it does not necessarily follow that, when two colloidal preparations of the same material from different sources show identical chemical composition, they should also show identical physical behavior, for it is altogether possible that one preparation lies much nearer the boundary between the crystalloidal and colloidal states of matter than does the other.

Wood (*16*) and Wood and Hardy (*18*) have shown that wheat gluten is an emulsoid colloid. All proteins which have been investigated belong to this class. One of the most characteristic reactions of the emulsoids is that they have a great affinity for water, being sometimes classified as "hydrophylic" colloids. The degree of this affinity for water may be altered by the addition of salts, acids, or alkalis to the dispersion medium.

Hofmeister (7) was among the first to study the conditions causing colloidal swelling of proteins and other workers have extended his observations. Fischer (*4*) has summarized the data of these workers and added extensive experiments of his own. It has been found that the addition of an acid or an alkali causes a hydrophylic colloid to imbibe more water, which, if the colloid is in the form of a gel, results in a swelling of the material, and that this swelling can be more or less completely inhibited by the addition of salts.

Wood appears to have been the first to attribute the differences between strong and weak flours to the physical properties of their proteins

[1] One of the latest of these résumés is that of Blish (*3*). In his paper the various researches are reviewed and criticised, and new data are added.

rather than to chemical difference in the flours. His first paper (*16*) is devoted to a study of possible chemical differences. He determined total nitrogen, total gliadin nitrogen, amid nitrogen in the gliadin, ratio of gliadin to total protein, total ash, total soluble ash, acid, and carbon-dioxid production in several different flours. He contends that the size of the loaf is regulated to a large extent by the fermentable sugars which are present, this being indicated by the carbon dioxid evolved, while—

> shapeliness, and probably gas retention, are dependent on the physical properties of gluten as modified by the presence of varying proportions of salts.

In later papers Wood (*17*) and Wood and Hardy (*18*, *19*) investigated certain of the physical properties of gluten, especially as to the effect of electrolytes in solution upon its physical state.

That the gluten which is washed from a strong flour is different in character from that obtained from a weak flour is a matter of general knowledge. Shutt (*13*, *p. 60*) visualizes the differences between such glutens as follows:

> In flours of high bread-making values the gluten is resilient, elastic, firm, and cohesive; in poor flours it may be flabby, nonresilient, soft, or sticky.

Wood suspended strings of gluten about the size of a pencil across V-shaped glass rods in beakers containing varying concentrations of different acids and then noted the concentration at which cohesion was so far reduced as to allow the gluten to fall off the rod and disperse in a cloudy solution. He apparently used gluten from only one flour in these experiments.

In this manner it was found that gluten suspended in distilled water retained its coherence almost indefinitely, but that in solutions of hydrochloric acid as dilute as *N/1,000* dispersion began almost immediately. This action increased with an increased concentration of acid up to about *N/30*, and then decreased again until at a concentration of approximately *N/12* the gluten became—

> permanently coherent and much harder and more elastic, and less sticky than in its original condition.

Similar experiments were conducted with sulphuric, phosphoric, oxalic, acetic, lactic, citric, and tartaric acids, both with and without the additions of certain salts. Unfortunately Wood does not give the necessary tabular data to permit exact comparisons; and in the curve shown, those for "acid alone" are omitted, and only certain of those for "acid + salt" are given. He finds, however, that the order in which the acids affected the coherence of the gluten was (1) hydrochloric, (2) sulphuric, (3) phosphoric, (4) oxalic. The three remaining acids behaved quite differently, for, while dilute solutions caused disintegration, this

became increasingly rapid with increasing concentrations of acid, and no practicable concentration could be found at which coherence reappeared.[1]

Wood likewise observed that the addition of salts to the acid solution counteracted in a large measure the effect of the acid. He therefore postulated (*17, p. 272*) that—

> the variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of the glutens themselves.

Wood and Hardy (*17, 18*) and Hardy (*6*) support this view in later papers.

Upson and Calvin (*14*) were the next to study the colloidal swelling of gluten. They employed a more exact technic than did Wood,[2] using the method employed by Hofmeister (*7*) in his investigations on the swelling of animal proteins. In their experiments the gluten was first freed from starch by washing it in a stream of distilled water. It was then pressed out between glass plates to a fairly uniform thickness and, after standing for some time, was cut into small disks. These disks were weighed to the nearest centigram, placed in beakers containing acid solutions of varying concentrations, and allowed to remain for a constant period of time. They were then removed, drained, and reweighed. The increase in weight due to imbibition of water was calculated to the amount imbibed per gram of moist gluten. The experiments were then repeated, except that a series of salts was added to the different concentrations of the acids. The addition of the salts caused a diminution of the water imbibition. They found that in dilute acids the gluten swells and—

> the disks puff up and take on an appearance somewhat resembling cotton balls, finally becoming transparent, soft, and gelatinous.

They furthermore found that the taking up and giving off of water was largely reversible and, by neutralizing the acid after swelling of the disks had taken place, it would lose water and again become a firm coagulum.

In a later publication (*15*) the same investigators give results of further studies on the colloidal swelling of wheat gluten as related to baking strength of flour and conclude that—

> strength is related to soluble acid and salt content of the flour. Flours containing acids and salts in such combinations as to favor water absorption will behave as "weak" flours, whereas those containing acids and salts in such combinations as inhibit water absorption will behave as strong flours when baked.

[1] Fischer (*4*) gives a different order for the effectiveness of acids causing swelling of animal proteins—that is, (1) hydrochloric, (2) phosphoric, (3) lactic, (4) formic, (5) oxalic, (6) nitric, (7) acetic, (8) citric, (9) sulphuric. The noteworthy differences between these two lists lies in the relative position of sulphuric acid.

[2] Wood allowed the gluten to imbibe water until it lost coherence and began to disperse as a sol. Upson and Calvin, on the other hand, determined the weight of the water imbibed by the gluten in a fixed period of time and before imbibition had progressed far enough to cause dispersion.

It should be noted that this finding is in many respects similar to that of Wood (*17*):

> The variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the glutens themselves.

There are, however, marked differences between these two statements; these will be considered later in this paper.

EXPERIMENTAL WORK

THE PROBLEM

In the experiments reported by Wood and in those recorded by Upson and Calvin it would appear that in each instance the action of acids and salts had been tested upon gluten derived from only one flour, and this presumably a "strong" flour, and that the application of such data to the problem of flour strength was made by analogy rather than actual observation. It was thought worth while, therefore, to repeat the work of Upson and Calvin (*14*, *15*), with glutens derived from flours of widely differing baking strength in order to determine what correlation, if any, exists between the baking qualities of the flour and the hydration capacity of the gluten.

MATERIAL USED

Five different flours were used. The first, a typical Minneapolis patent grade milled from No. 1 northern hard spring wheat from the 1916 crop. The second was a first clear grade milled from the 1915 crop. The three other samples were milled in the State of Oregon from typical soft wheats grown in that section, and are "straight-grade" flours. It is to be regretted that sufficient amounts of two of the western flours, W_1 and W_2, were not available for all of the experimental work.

METHOD OF EXPERIMENTATION

The method used in studying these flours was the same as that used by Hofmeister in his work on the swelling of gelatin and, as mentioned above, by Upson and Calvin. Briefly, it consisted in first doughing 200 gm. of flour by adding the required amount of distilled water. The dough was then permitted to stand for from 30 minutes to an hour under distilled water, after which it was washed for 15 minutes under a stream of distilled water. Almost all of the starch was washed out in this manner. The gluten was then submerged under distilled water until all the desired samples of gluten had been prepared.

It was interesting to note the difference in the character of the gluten prepared from the different samples. The patent flour, from No. 1 northern grade of wheat, called "P," gave a rather firm coherent gluten, as did the first clear grade, "C." The three western flours, designated "W^1," "W^2," and "W^3," gave a more friable, sticky, and less coherent gluten which was much more difficult to obtain than was the gluten from

the two other flours, so greatly was it lacking in coherency. The gluten from each of these flours was then pressed out to a nearly uniform thickness of about 3 mm. between glass plates, and after draining for a few minutes (this interval of time was kept as nearly constant as possible), was cut into small disks by means of a large cork borer. These disks, which were fairly uniform as to size, shape, and weight, were weighed out to an accuracy of 5 mgm., placed in acid solutions of varying compositions and concentrations, and left for exactly 50 minutes. They were then removed, drained for about 10 minutes on a perforated porcelain plate and reweighed. The change in weight was calculated to the change per gram of moist gluten. Preliminary experiments were undertaken in order to determine the maximum time during which glutens

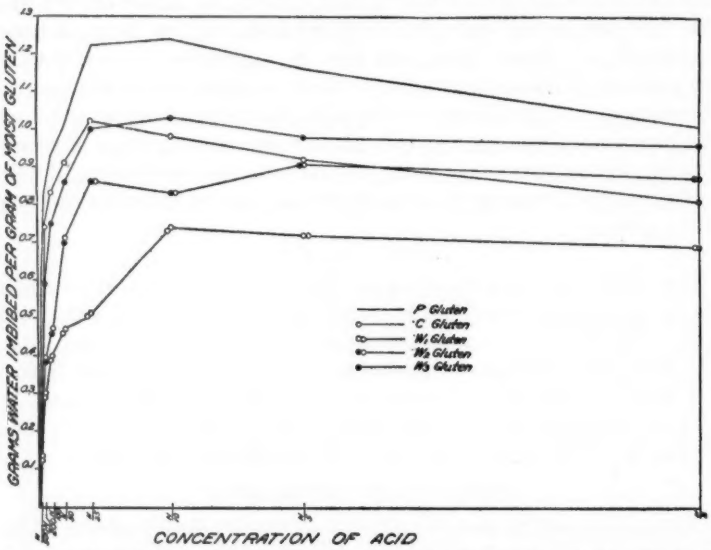

FIG. 1.—Graph showing the imbibition curves for the various glutens in different concentrations of lactic acid.

from all the flours could remain submerged in the different concentrations of the various acids and still retain their coherence sufficiently to make weighing possible. This time was found to be 50 minutes. In order that the results with the different flours might be comparable, this time interval was kept exactly the same in all the experiments reported in the following tables. The glutens for any given set of experiments were prepared at the same time and placed in acids at the same temperature. Every possible precaution was taken to eliminate variations in experimental conditions, and we believe that any appreciable difference between the recorded constants may be attributed to intrinsic differences in the various glutens themselves.

This method of measuring water imbibition or "hydration capacity" is obviously somewhat crude when compared with the usual chemical

procedure, but the errors are reduced to a minimum by taking the average of several different determinations.

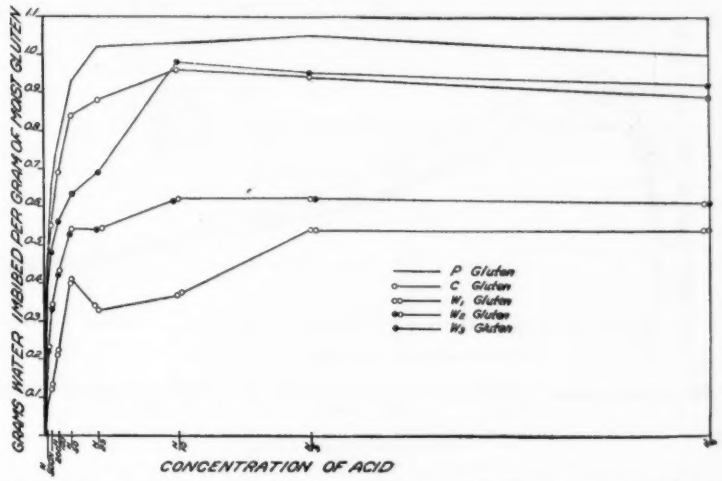

FIG. 2.—Graph showing the imbibition curves for the various glutens in different concentrations of acetic acid.

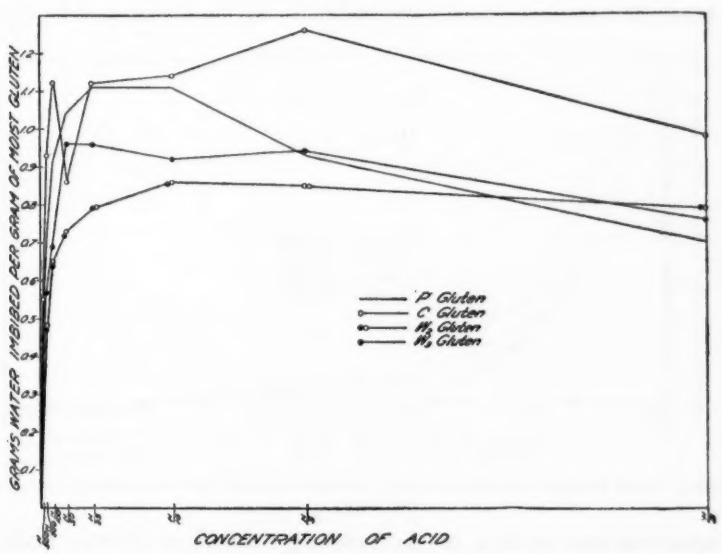

FIG. 3.—Graph showing the imbibition curves for the various glutens in different concentrations of orthophosphoric acid.

It will be observed that the figures in Tables I to V are all average values of from three to seven individual determinations. It was not thought worth while to record all of the individual values. The agree-

ment between duplicates was, however, of the same order as that shown in Upson and Calvin's tables (*14*, *15*).

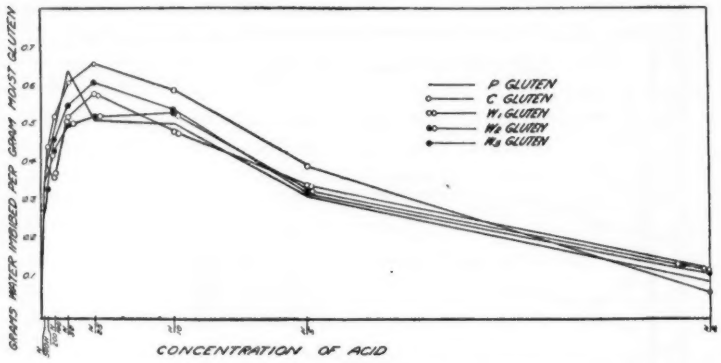

FIG. 4.—Graph showing the imbibition curves for the various glutens in different concentrations of oxalic acid.

EXPERIMENTAL DATA

(a) IMBIBITION OF WATER BY THE DIFFERENT GLUTENS IN THE PRESENCE OF DILUTE ACIDS.—The amount of water imbibed by the different glutens under the above conditions has been measured in eight

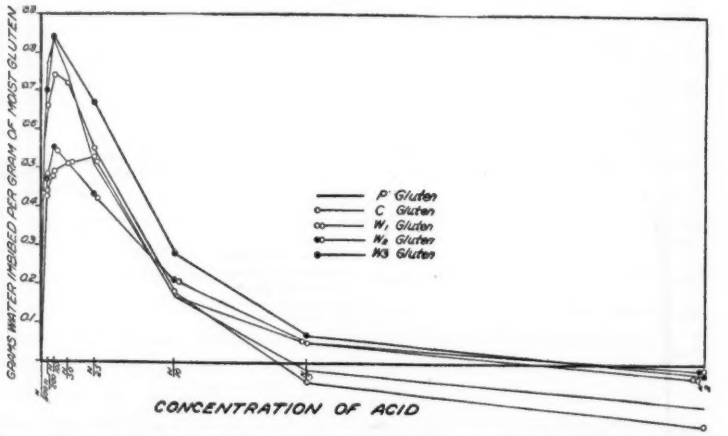

FIG. 5.—Graph showing the imbibition curves for the various glutens in different concentrations of hydrochloric acid.

concentrations of each of the following acids: hydrochloric, oxalic, orthophosphoric, lactic, acetic, and boric. The average data obtained from these experiments are shown in the first five columns of Tables I to IV and in Table V. The results are shown graphically in figures 1 to 5. The values for boric acid have not been plotted, inasmuch as it appears that boric acid has little or no effect on water imbibition by gluten.

TABLE I.—*Relative imbibition of the glutens from patent, clear, and western flours in lactic acid and in lactic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).																							
	Lactic acid alone.				Lactic acid + $\frac{M}{200}$ potassium chlorid.				Lactic acid + $\frac{M}{200}$ potassium phosphate.			Lactic acid + $\frac{M}{200}$ potassium tartrate.			Lactic acid + $\frac{M}{200}$ calcium chlorid.			Lactic acid + $\frac{M}{200}$ mercuric chlorid.			Lactic acid + $\frac{M}{200}$ aluminium sulphate.			Lactic acid + $\frac{M}{200}$ magnesium sulphate.		
	W_1.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.
None.....	0.00	−0.01	−0.01	0.01	−0.01	−0.02	−0.24	−0.04	−0.08	−0.13	−0.05	0.04	0.05	0.07	−0.08	−0.16	−0.03	0.56	0.50	0.31	0.06	0.01	0.09	−0.08	−0.20	−0.04
N/500.....	.12	.23	.63	.65	.45	.22	.14	.18	.22	.22	.21	.10	.10	.15	.02	.07	.09	.44	.49	.21	.09	.05	.12	.06	.18	.00
N/200.....	.29	.38	.82	.74	.59	.38	.39	.33	.38	.31	.32	.31	.35	.24	.25	.30	.25	.42	.47	.19	.15	.14	.14	.03	.07	.07
N/100.....	.40	.46	.93	.83	.75	.55	.55	.39	.50	.67	.54	.41	.54	.36	.42	.43	.38	.37	.47	.18	.22	.26	.17	.12	.09	.13
N/50.....	.47	.71	1.10	.91	.86	.66	.65	.54	.63	.76	.62	.73	.74	.48	.48	.56	.43	.37	.30	.15	.28	.33	.24	.23	.29	.23
N/25.....	.51	.86	1.22	1.02	1.00	.79	.73	.61	.77	.79	.78	.77	.81	.65	.60	.57	.54	.33	.25	.13	.36	.38	.31	.35	.44	.30
N/10.....	.74	.83	1.24	.98	1.03	.94	.90	.75	.83	.93	.88	.92	.85	.74	.73	.72	.64	.30	.27	.14	.46	.39	.35	.53	.52	.41
N/5.....	.71	.91	1.16	.92	.98	1.05	.85	.85	.90	.97	.83	.95	1.11	.81	.83	.83	.73	.32	.20	.16	.54	.55	.43	.66	.59	.48
N/2.....	.69	.87	1.01	.81	.96	.96	.78	.83	.93	.93	.81	1.01	.91	.76	.83	.83	.94	.33	.19	.10	.57	.58	.44	.64	.49	.56

[1] The figures for P and C flours in the acid alone controls are the averages of six different determinations; those for W_1 and W_2 are the averages of three; and those for W_3 are the averages of seven different determinations. The figures for acid plus the various salts are in every case averages of three different determinations.

TABLE II.—*Relative imbibition of the glutens from patent, clear, and western flours in hydrochloric acid and in hydrochloric acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).																							
	Hydrochloric acid alone.					Hydrochloric acid + $\frac{M}{200}$ potassium chlorid.			Hydrochloric acid + $\frac{M}{200}$ potassium phosphate.			Hydrochloric acid + $\frac{M}{200}$ potassium tartrate.			Hydrochloric acid + $\frac{M}{200}$ calcium chlorid.			Hydrochloric acid + $\frac{M}{200}$ mercuric chlorid.			Hydrochloric acid + $\frac{M}{200}$ aluminium sulphate.			Hydrochloric acid + $\frac{M}{200}$ magnesium sulphate.		
	W_1.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.	P.	C.	W_2.
None.....	−0.03	0.01	−0.02	−0.06	0.00	−0.09	−0.13	−0.03	−0.09	−0.07	−0.03	0.04	0.07	0.09	−0.05	−0.18	−0.01	0.66	0.50	0.26	0.02	0.02	0.06	−0.05	−0.24	−0.04
N/500.....	.27	.34	.57	.59	.52	.31	.20	.24	.35	.33	.33	.16	.18	.21	.24	.16	.26	.53	.34	.35	.10	.10	.10	.01	.13	.04
N/200.....	.44	.47	.77	.66	.70	.42	.39	.43	.58	.49	.49	.30	.34	.35	.41	.38	.45	.58	.56	.36	.19	.22	.17	.13	.08	.17
N/100.....	.49	.55	.83	.74	.84	.67	.51	.55	.66	.61	.62	.38	.56	.53	.51	.59	.54	.69	.61	.55	.29	.35	.24	.27	.23	.22
N/50.....	.51	.51	.74	.72	.81	.68	.55	.67	.67	.63	.63	.59	.58	.61	.46	.40	.45	.62	.65	.60	.38	.36	.35	.34	.33	.35
N/25.....	.53	.43	.51	.55	.67	.49	.42	.50	.57	.59	.56	.47	.51	.52	.48	.51	.47	.38	.32	.45	.34	.39	.31	.32	.28	.32
N/10.....	.17	.21	.17	.18	.28	.17	.12	.21	.19	.14	.22	.16	.19	.24	.15	.06	.20	.08	.03	.12	.15	.15	.16	.18	.04	.18
N/5.....	.05	.05	−.02	−.05	.07	−.01	−.08	.04	−.01	−.09	.05	.02	.04	.04	.00	−.23	.04	−.02	−.07	.02	−.03	−.05	.07	−.03	−.03	.04
N/2.....	−.04	−.02	−.11	−.16	−.03	−.13	−.22	−.04	−.18	−.25	−.07	−.05	−.08	−.03	−.10	−.34	−.03	−.07	−.18	−.03	−.07	−.20	−.03	−.06	−.30	−.03

[1] These figures are the averages of the same number of determinations in each case as with the corresponding columns in Table I.

TABLE III.—*Relative imbibition of the glutens from patent, clear, and western flours in acetic acid and in acetic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).													
	Acetic acid alone.					Acetic acid + $\frac{M}{200}$ potassium chlorid.			Acetic acid + $\frac{M}{200}$ potassium phosphate.			Acetic acid + $\frac{M}{200}$ potassium tartrate.		
	W_1	W_2	P	C	W_3	P	C	W_3	P	C	W_3	P	C	W_3
None....	0.01	0.00	0.01	0.01	0.03	−0.10	−0.14	−0.06	−0.04	−0.04	−0.04	0.02	−0.08	0.09
N/500...	.08	.22	.48	.45	.39	.07	.06	.11	.18	.21	.18	.05	.03	.12
N/200...	.12	.33	.65	.65	.48	.20	.21	.24	.28	.29	.32	.13	.12	.17
N/100...	.21	.42	.76	.69	.56	.30	.42	.30	.39	.37	.29	.24	.31	.24
N/50....	.41	.54	.93	.84	.63	.42	.67	.37	.41	.48	.38	.30	.51	.30
N/25....	.33	.54	1.02	.88	.69	.51	.71	.49	.54	.56	.45	.49	.56	.36
N/10....	.37	.62	1.03	.96	.98	.58	.76	.55	.66	.64	.55	.65	.62	.46
N/5.....	.54	.62	1.05	.94	.95	.62	.75	.64	.77	.68	.60	.62	.75	.53
N/2.....	.54	.61	1.00	.89	.92	.72	.72	.64	.77	.69	.65	.74	.80	.60

[1] The figures for P and C flours in the acid alone controls are the averages of six different determinations. All of the remaining in both acid alone, and acid plus salts are averages of three different determinations.

TABLE IV.—*Relative imbibition of the glutens from patent, clear, and western flours in oxalic acid and in oxalic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).													
	Oxalic acid alone.					Oxalic acid + $\frac{M}{200}$ potassium chlorid.			Oxalic acid + $\frac{M}{200}$ potassium phosphate.			Oxalic acid + $\frac{M}{200}$ potassium tartrate.		
	W_1	W_2	P	C	W_3	P	C	W_3	P	C	W_3	P	C	W_3
None.....	−0.01	−0.01	−0.02	−0.03	0.02	−0.11	−0.11	−0.04	−0.04	−0.10	−0.08	0.03	−0.02	0.08
N/500.....	.23	.27	.33	.29	.26	.12	.17	.16	.21	.16	.20	.08	.04	.11
N/200.....	.40	.38	.42	.45	.34	.22	.24	.24	.38	.32	.31	.14	.14	.18
N/100.....	.37	.44	.50	.53	.47	.36	.38	.38	.51	.40	.42	.31	.37	.24
N/50.....	.53	.51	.65	.62	.56	.47	.50	.47	.64	.54	.55	.44	.49	.40
N/25.....	.59	.53	.52	.67	.62	.53	.63	.48	.70	.70	.64	.53	.71	.51
N/10.....	.49	.54	.51	.60	.55	.41	.49	.44	.61	.63	.58	.53	.61	.49
N/5.....	.35	.34	.32	.40	.33	.26	.22	.24	.37	.35	.35	.32	.48	.32
N/2.....	.13	.13	.10	.07	.12	.06	.05	.11	.12	.09	.13	.11	.17	.15

[1] These figures are the average of the same number of determinations in each case as with the corresponding columns in Table III.

TABLE V.—*Relative imbibition of the glutens from patent, clear, and western flours in ortho-phosphoric and boric acids.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).							
	Ortho-phosphoric acid.				Boric acid.			
	P	C	W_2	W_3	P	C	W_2	W_3
None.....	−0.02	0.03	−0.04	−0.01	−0.02	−0.10	−0.01	−0.02
N/500.....	.53	.55	.18	.40	− .04	− .10	.00	− .02
N/200.....	.71	.93	.47	.57	− .05	− .06	− .01	− .01
N/100.....	.92	1.12	.65	.69	− .05	− .05	− .02	.01
N/50.....	1.04	.86	.73	.96	− .05	− .06	− .02	− .01
N/25.....	1.11	1.12	.79	.96	− .05	− .07	− .02	− .02
N/10.....	1.11	1.14	.86	.92	− .04	− .06	− .01	− .02
N/5.....	.93	1.26	.85	.94	− .04	− .08	− .01	.00
N/2.....	.70	.98	.79	.76	− .05	− .09	− .01	− .04

[1] These figures are the averages of three different determinations.

ANTAGONISTIC ACTION OF SALTS UPON THE IMBIBITION OF WATER BY THE VARIOUS GLUTENS IN THE PRESENCE OF HYDROCHLORIC, OXALIC, LACTIC, AND ACETIC ACIDS.—The amount of water imbibed by the P, C, and W_3 glutens was again measured as under (a), with the exception that various salts in 0.005 molar concentration were added to the acid solutions. The salts used were potassium chlorid (KCl), potassium phosphate (KH_2PO_4), potassium tartrate ($KHC_4H_4O_6$), calcium chlorid ($CaCl_2+2H_2O$), mercuric chlorid ($HgCl_2$), magnesium sulphate ($MgSO_4+7H_2O$), and aluminium sulphate $Al_2(SO_4)_3$. All of these salts were used with hydrochloric and lactic acids, but only the first three with oxalic and acetic

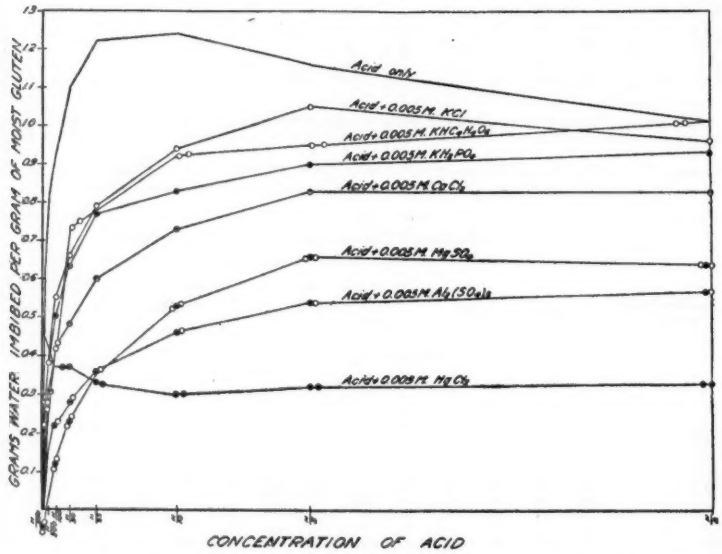
GRAMS WATER IMBIBED PER GRAM OF MOIST GLUTEN
CONCENTRATION OF ACID
Acid only
Acid + 0.005 M. KCl
Acid + 0.005 M. KHC4H4O6
Acid + 0.005 M. KH2PO4
Acid + 0.005 M. CaCl2
Acid + 0.005 M. MgSO4
Acid + 0.005 M. Al2(SO4)3
Acid + 0.005 M. HgCl2

FIG. 6.—Graph showing the imbibition curves for P gluten in lactic acid and in lactic acid plus certain salts.

acids. The quantity of water, in grams, imbibed per gram of moist gluten under these conditions is given in the latter columns of Tables I to IV. It is physically impossible to visualize the data given in these tables without the aid of graphs. The corresponding curves were accordingly constructed and are shown in figures 6 to 17.

(c) FLOUR ANALYSES AND BAKING TESTS.—In Table VI are shown the following data determined from either the analysis of the flour or from baking tests: Ash on dry flour as determined by ignition in platinum in a muffle furnace, ash in an aqueous extract of the flour,[1] ratio of "soluble ash" to

[1] 100 c.c. of carbon-dioxid-free water containing a few drops of toluene were added to 25 gm. of flour; the mixture was thoroughly stirred and allowed to stand for three hours, after which the solution was filtered off for the "soluble-ash" and specific-conductivity determinations.

"total ash" in the flours, percentage of wet gluten and of dry gluten in the flours, percentage of ash in the dry gluten, specific conductivity of the flour extract at 25° C., water added to make the dough, volume of loaf, texture of loaf, and expansimeter test (*2*).

TABLE VI.—*Relative flour analyses and baking tests on P, C, W_1, W_2, and W_3 flours*

Mark.	Ash.		Soluble ash.	Crude gluten.		Ash in dry gluten.	Specific conductivity.	Water used per 100 gm. of flour.	Volume of loaf.	Texture.	Expansimeter test.
	Dry matter.	Water extract.	Total ash.	Wet.	Dry.						
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*		*C. c.*	*C. c.*		*C. c.*
P	0.46	0.27	59.32	30.23	10.02	0.31	1.08×10^{-3}	60.0	1,440	100	870
C	.56	.33	58.78	32.39	10.57	.32	1.22×10^{-3}	58.2	1,405	95	775
W_1	.61	.40	64.22	24.11	7.68	.55	1.46×10^{-3}	56.0	1,345	97	650
W_2	.62	.37	55.40	19.56	6.66	.42	1.29×10^{-3}	57.1	1,220	96	620
W_3	.49	.30	61.00	25.45	7.28	.39	1.04×10^{-3}	57.5	1,320	95	620

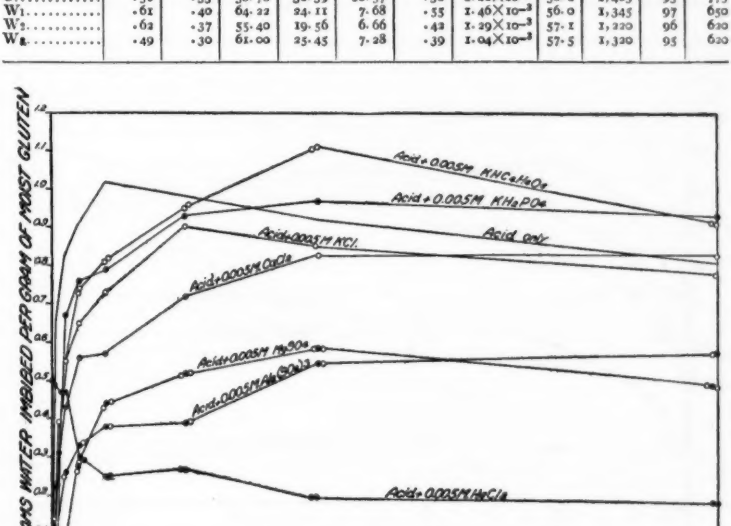

FIG. 7.—Graph showing the imbibition curves for C gluten in lactic acid and in lactic acid plus certain salts.

The notes taken during the progress of the baking test are as follows:

W_3 rose much more slowly in the expansimeter. In No. W_1, W_2, and W_3 the loaf was to some extent too compact and solid, apparently because of the fact that the gluten was not sufficiently tenacious to retain the carbon dioxid against the weight of the loaf above it. This was especially marked in W_2, where the lower third of the loaf was almost entirely lacking in porosity and light, uniform, velvety appearance.

RELATION BETWEEN THE QUALITY OF THE VARIOUS GLUTENS AND THEIR DEGREE OF HYDRATION

A study of the preceding tables and graphs confirms certain of the findings of Upson and Calvin, that—

gluten is an emulsoid colloid and shows all the properties of this class of compounds

and that—

gluten absorbs water from dilute acid solutions, thereby losing its tenacity and ductility, becoming soft and gelatinous. The presence of small amounts of neutral salts in the dilute acid solutions inhibits water absorption by gluten.

The data, however, do not support the latter part of their third statement that—

the bread-making qualities of dough made from wheat flours are dependent on the quantity and quality of the contained gluten. Quality of gluten is regulated by the

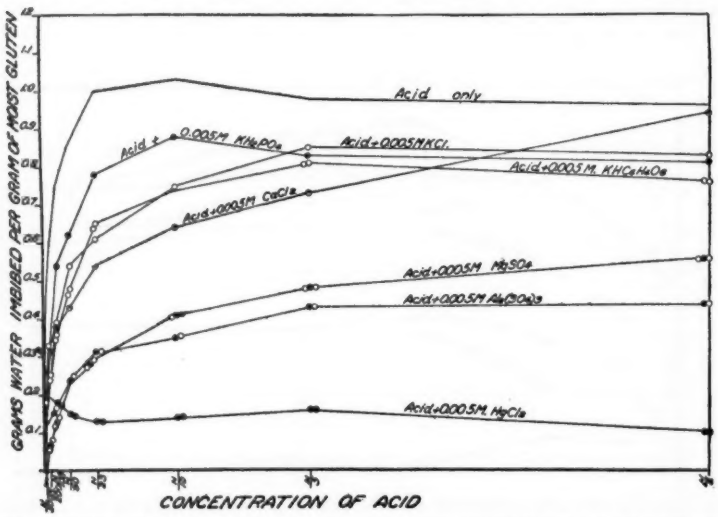
GRAMS WATER IMBIBED PER GRAM OF MOIST GLUTEN
CONCENTRATION OF ACID
Acid only
Acid + 0.005 M KH2PO4
Acid + .0005 M KCl
Acid + .0005 M KHC4H4O6
Acid + .0005 M CaCl2
Acid + .0005 M MgSO4
Acid + .0005 M Al2(SO4)3
Acid + .0005 M MgCl2

FIG. 8.—Graph showing the imbibition curves for W1 gluten in lactic acid and in lactic acid plus certain salts.

kind and concentration of the acids and salts present in the dough. If the kinds and amounts of the acids and salts are such as to favor water absorption, the quality of the gluten will be poor, whereas the presence of acids and salts in such amounts as tend to inhibit water absorption makes for an improved gluten,

but, on the contrary, all the evidence is directly opposed to such a conclusion.

The above statements by Upson and Calvin seem to have only one interpretation: that a weak gluten is weak because it is hydrated to a greater extent than is a strong gluten, and that in this respect, and in this respect only, does a weak gluten differ from a strong gluten. Upson and Calvin present data in their own bulletin which would have refuted this idea had they made the necessary calculations.

In Table VI are presented the percentages of moist gluten and dry gluten in the five flours which we have studied. If now we use these figures to calculate the percentage of water in the moist gluten, or, in other words, the amount of water imbibed by the dry gluten in preparing the gluten for the tests, we find the figures given in Table VII.

TABLE VII.—*Percentage of water in the moist gluten of various flours*

Mark.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to P.	Difference of actual from hypothetical water content.
P	66.85	33.15	.....	.....
C	67.37	32.63	65.82	+ 1.55
W_1	68.15	31.85	64.24	+ 3.89
W_2	66.00	34.00	68.56	− 2.56
W_3	71.39	28.61	57.69	+13.70

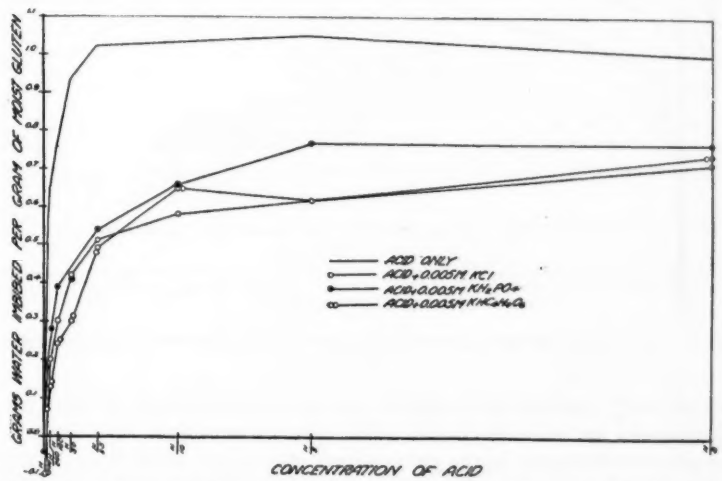
GRAMS WATER IMBIBED PER GRAM OF MOIST GLUTEN
CONCENTRATION OF ACID
ACID ONLY
ACID + 0.005 M KCl
ACID + 0.005 M KH2PO4
ACID + 0.005 M KHC4H4O6

FIG. 9.—Graph showing the imbibition curves for P gluten in acetic acid and in acetic acid plus certain salts.

Expressing these figures in the conventional form of grams of water imbibed per gram of moist gluten and using the patent flour P as our standard, we find that—

C showed an excess of 0.015 gm. of water per gram of moist gluten over P.
W_1 showed an excess of 0.039 gm. of water per gram of moist gluten over P.
W_2 showed a deficiency of 0.026 gm. of water per gram of moist gluten over P.
W_3 showed an excess of 0.137 gm. of water per gram of moist gluten over P.

The figures for C, W_1, and W_2 are certainly within experimental error and as such can have no significance; we must therefore conclude that

these three glutens, although differing widely in "quality" and in physical properties from P, were hydrated to almost exactly the same extent as was P. However, the difference in moisture content of W_3 gluten is probably significant. It is necessary, therefore, to consider what effect this increased moisture content should have in the experiments in Tables I to V, provided that the gluten had originally possessed the same physical properties as P. W_3 contains 7.28 per cent of dry gluten. The dry gluten of P composes 33.15 per cent of the wet gluten; therefore the wet gluten of W_3 should weigh 21.96 gm. at the same hydration as the gluten of P. It actually did weigh 25.45 gm., or an excess of 3.49 gm. of water based on a weight of 21.96 gm. of moist gluten, this indicating an imbibition of 0.16 gm. of water per gram of moist gluten (of P quality). This amount is entirely too small to account

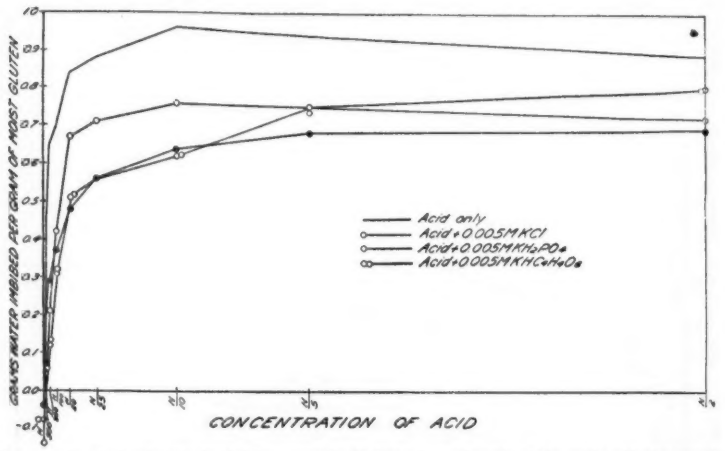
GRAMS WATER IMBIBED PER GRAM OF MOIST GLUTEN
CONCENTRATION OF ACID
Acid only
Acid + 0.005 M KCl
Acid + 0.005 M KH2PO4
Acid + 0.005 M KHC4H4O6

FIG. 10.—Graph showing the imbibition curves for C gluten in acetic acid and in acetic acid plus certain salts.

for the very marked differences in the physical properties of the two glutens and the only conclusion which remains possible is that there is an inherent difference in the glutens from strong and weak flours, that the colloidal properties of the glutens from the different flours are not identical and would not be identical even if the flours had the same salt and acid content.

These data are substantiated by the moist and dry gluten figures presented by Upson and Calvin, although these authors, as noted above, expressed the opinion that the difference between a strong and a weak gluten was due to its degree of hydration. Under Table XII on page 23 of their bulletin (*15*) are given the percentages of wet and dry gluten from several different mill streams of flour. Using carbon-dioxid-free water as the washing agent, they give the percentages of wet and dry gluten as follows:

Flour.	Wet gluten.	Dry gluten.
	Per cent.	*Per cent.*
First middlings	23.5	7.1
Third middlings	27.8	8.7
Fifth middlings	33.7	11.2
Seventh middlings	36.01	12.4

By making the same calculations on these different samples of gluten as was done on our own samples we have the results given in Table VIII.

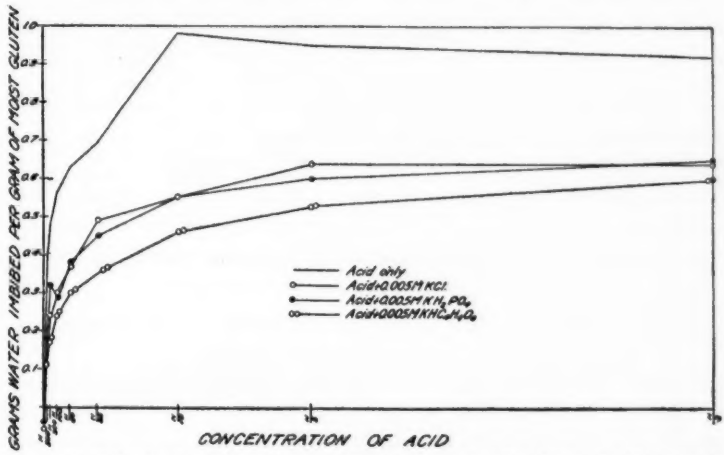

FIG. 11.—Graph showing the imbibition curves for W3 gluten in acetic acid and in acetic acid plus certain salts.

TABLE VIII.—*Percentage of water in the moist gluten from flours washed with carbon-dioxid-free water*

Flour.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to first middlings.	Difference of actual from hypothetical water content.
First middlings	69.8	30.2	.....	.....
Third middlings	68.7	31.3	72.34	−3.64
Fifth middlings	66.7	33.3	76.96	−10.26
Seventh middlings	65.1	34.9	80.66	−15.56

Again expressing these figures in grams of water absorbed per gram of moist gluten and using the first middlings as the standard, we find that—

third middlings shows a deficiency of 0.036 gm. of water per gram of moist gluten over first middlings; fifth middlings shows a deficiency of 0.103 gm. of water per gram

of moist gluten over first middlings; seventh middlings show a deficiency of 0.156 gm. of water per gram of moist gluten over first middlings.

When a 0.5 per cent sodium-chlorid solution was used as the washing agent, Upson and Calvin obtained wet and dry gluten figures which, when used for calculation, give the results of Table IX.

TABLE IX.—*Percentage of water in moist gluten from flours washed with a 0.5 per cent sodium-chlorid solution*

Flour.	Wet gluten.	Dry gluten.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to first middlings.	Difference of actual from hypothetical water content.
First middlings	32.1	9.4	70.7	29.3	.....	.....
Third middlings	34.5	10.3	70.1	29.9	72.2	−2.1
Fifth middlings	37.5	11.2	70.1	29.9	72.2	−2.1
Seventh middlings	38.6	12.0	68.9	31.1	75.0	−6.1

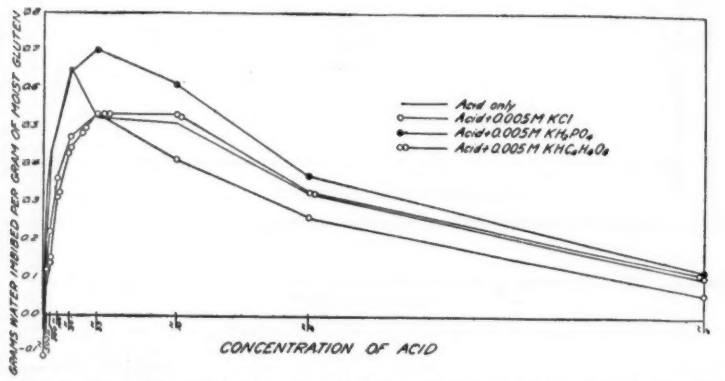

FIG. 12.—Graph showing the imbibition curves for P gluten in oxalic acid and in oxalic acid plus certain salts.

These figures, expressed again in grams of water absorbed per gram of moist gluten with the first middlings as the standard, give the following:

Third middlings shows a deficiency of 0.021 gm. of water per gram of moist gluten over first middlings; fifth middlings shows a deficiency of 0.021 gm. of water per gram of moist gluten over first middlings; seventh middlings shows a deficiency of 0.061 gm. of water per gram of moist gluten over first middlings.

Upson and Calvin found that the seventh middlings gave results similar to a "low-grade" flour the gluten from which, according to their theory, we should expect to find hydrated to a greater degree than that from the first middlings. Such, however, is not the case. In both distilled water and in 0.5 per cent sodium chlorid the "low-grade" gluten

appears to be less hydrated than is the strong gluten. To be sure, four of the six differences are probably within the experimental error, but it is possibly significant that each of the six determinations shows a negative sign. Thus, their own data refute their idea that quality of gluten is regulated by the degree of imbibition and upholds our contention that quality of gluten is not determined by the amount of acids and salts in

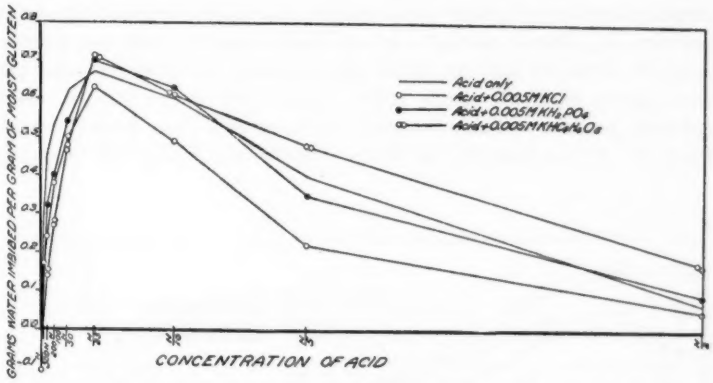

FIG. 13.—Graph showing the imbibition curves for C gluten in oxalic acid and in oxalic acid plus certain salts.

the flour, but by the physico-chemical nature of the colloids comprising the gluten.

Again, these authors list data for a "patent" and a "low-grade" flour in Table XI of their bulletin. These data when recalculated give the following figures:

Flour.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if low-grade gluten were equal to "patent."	Difference between actual and hypothetical water content.
Patent	67.9	32.1	.....	.....
Low-grade	67.5	32.5	68.75	−1.25

The low-grade shows a deficiency of 0.0125 gm. per gram of moist gluten over the patent gluten. This result is within the experimental error of the "patent" grade, but certainly does not support their theory as to the cause of strong and weak glutens.

We have devoted considerable space to the theory of Upson and Calvin because of its important bearing in a study such as we have made, and we believe that we have presented sufficient evidence to show that it has no supporting evidence either in our own work or in any of their publications.

On the contrary, the data in both our tables and in their own are such as to prove the fallacy of their contention and to cause the theory to be definitely discarded.

Guthrie (5) concluded that the property of absorbing water, which a flour possesses, was dependent on the physical nature of the gluten present in the flour rather than upon the absolute quantity of the gluten. He suggests that this physical difference may be due to the relative proportions of glutenin and gliadin in the gluten, inasmuch as he found that dry glutenin will absorb nearly twice as much water as will dry gliadin. However, when his data are recalculated it is at once apparent that the moist gluten from the flours milled from "good bread wheats" were hydrated to almost exactly the same extent as were those from the weak flours, the glutens from the two flours of the former class of the 1896 crop

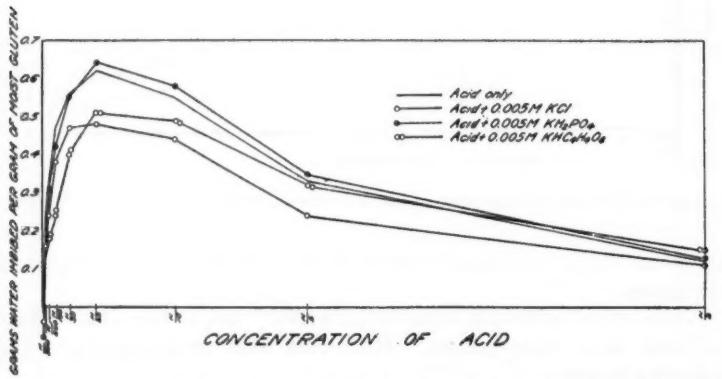

FIG. 14.—Graph showing the imbibition curves for W_2 gluten in oxalic acid and in oxalic acid plus certain salts.

containing 68.19 and 68.53 per cent of water and those from the weak flours 66.44 and 69.10 per cent. Similarly, for the 1895 flours, the strong flour gave 65.58 per cent of water in the moist gluten, while the glutens from three weaker flours contained 63.37, 64.80, and 64.51 per cent of water, respectively. There are certainly no differences in either set of figures large enough to account for the wide differences which were observed in the physical properties of the glutens. Neither do these figures tend to support Guthrie's conclusion that strength is regulated by the glutenin to gliadin ratio.

Turning now to our own data as given in Tables I to IV and in figures 1 to 5, inclusive, we find two noteworthy differences between the strong and weak glutens. These are (a) rate of hydration and (b) maximum capacity for hydration.

RATE OF HYDRATION

It will be observed that the different glutens, prepared and treated in exactly the same manner and for exactly the same interval of time, differ widely in their rate of hydration. For example, in Table I it is shown that 1 gm. of moist gluten from P flour in *N*/50 lactic acid imbibed 1.10 gm. of water, while W_1 gluten absorbed only 0.47 gm.; in *N*/500 acid the figures are, respectively, 0.63 and 0.12 gm. These examples are typical of other experiments in the tables, and when the graphs are inspected, they are so convincing that only one conclusion seems possible—that is,

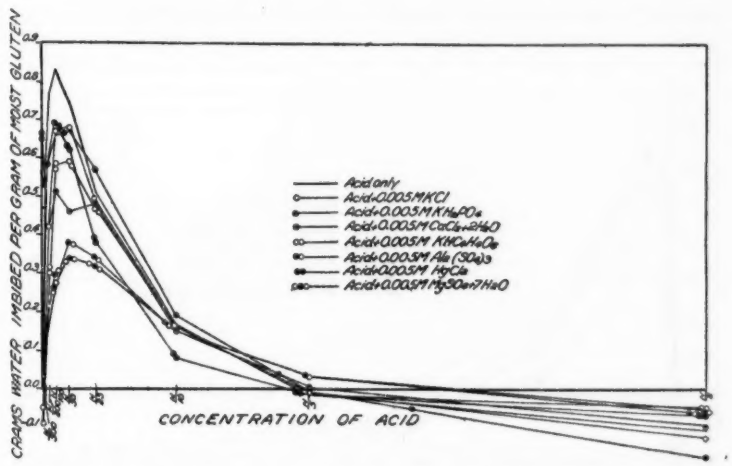

FIG. 15.—Graph showing the imbibition curves for P gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

that a weak gluten has a much lower rate of hydration than a strong gluten.

MAXIMUM CAPACITY FOR HYDRATION

There is, moreover, a marked difference in the maximum degree of hydration of the different glutens. In preliminary experiments it was found that disks of P gluten would retain their coherency and plasticity for as long as two hours in the different concentrations of lactic acid and still be so cohesive that they could be easily removed from the acid solutions by means of small forceps, although they had swelled to three or four times their former size and had imbibed as much as 2.22 gm. of water per gram of moist gluten.

On the other hand, the weak glutens W_1, W_2, and W_3 became so badly dispersed when immersed in the concentrations of acids causing maximum imbibition that in many instances they could not be collected for weighing in even so short a time as one hour, although even at this point

the weight of water absorbed must have been far below the quantity which the P gluten could imbibe and still remain coherent. We are forced to conclude, therefore, that not only does the weak gluten have a lower rate of imbibition than the strong gluten, but that it also has a much lower maximum hydration capacity; or, in other words, a gluten from a "weak" flour changes from a gel to a sol at a much lower degree of hydration than does gluten from a strong flour.

In short, the difference between a strong and a weak gluten is that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoid gels, and that of a colloidal gel in which these properties are much less marked.

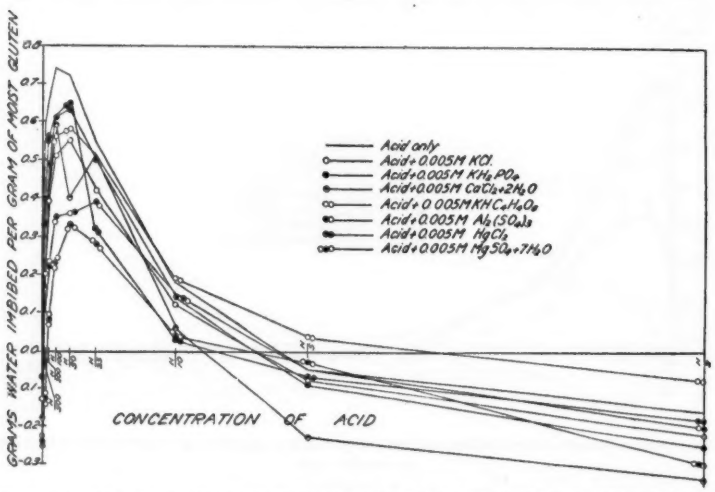

FIG. 16.—Graph showing the imbibition curves for C gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

COMPARATIVE MEASUREMENTS OF THE HYDRATION CAPACITY OF THE DIFFERENT GLUTENS IN VARIOUS CONCENTRATIONS OF LACTIC, ACETIC, PHOSPHORIC, OXALIC, AND HYDROCHLORIC ACIDS

It will be observed that the curves in figures 1, 2, and 3 are very different in form from those of figures 4 and 5. Upson and Calvin (*14*) observed similar differences between the curves for lactic and acetic acids and that for hydrochloric acid.

Both hydrochloric and oxalic acids are highly ionized when compared with the other acids,[1] although phosphoric acid has a dissociation con-

[1] Abbott and Bray (*1, p. 760*) give the following ionization constants: Ortho-phosphoric acid ($H_2PO_4^- + H^+$) 1.1×10^{-2}, ($HPO_4^- + H^+$) 1.95×10^{-7}, acetic acid 1.8×10^{-5}, and boric acid 1.7×10^{-9}. They give the constant for hydrochloric acid as 6×10^{-1}. This is possibly a typographical error, for Noyes (*12, p. 860*) gives the ionization constant for hydrochloric acid as 1, with those for acetic and phosphoric acids of the same value as given by Abbott and Bray. Landolt-Börnstein (*11, p. 1147*) give constants for oxalic and lactic acids as 3.8×10^{-2} and 1.38×10^{-4}, respectively.

stant more nearly like that of oxalic than like those of acetic or lactic acids.

At first glance, leaving the phosphoric-acid graphs out of consideration, it would appear that the amount of imbibition might be regulated solely by the hydrogen-ion concentration, but when we take into consideration the data for both phosphoric and boric acids, one of which is a relatively strong acid but which nevertheless produces the form of imbibition curve typical of much weaker acids and the other a very weak acid which produces no appreciable change in the degree of imbibition of the glutens, it becomes more and more improbable that the hydrogen-ion concentration is the only factor involved. Fischer (*4*, *p.* 44) has already come to a similar conclusion while studying the imbibition of water by animal proteins or tissues in solutions of acids, for he found that a "strong"

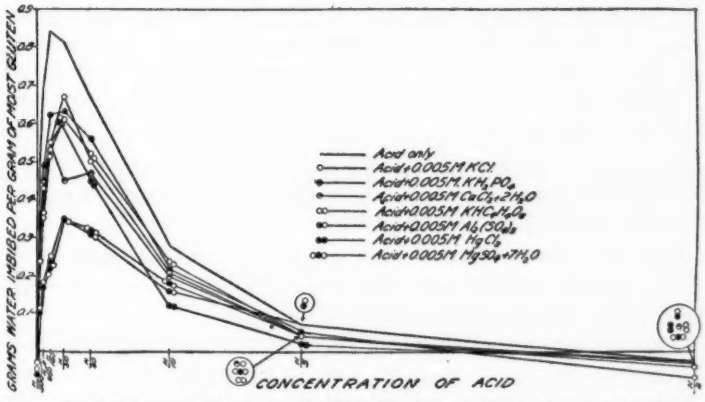

FIG. 17.—Graph showing the imbibition curves for W_3 gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

acid (hydrochloric) stands at the top of the list, another (sulphuric) stands at the very bottom, while a series of "weak" organic acids are found between.

Jessen-Hansen (*10*) has made an extensive study as to the relationship between the hydrogen-ion concentration of the flour and the volume and quality of the resulting loaf. He finds that there is an optimum hydrogen-ion concentration of about $P = .5$. For strong flours the optimum may slightly exceed this, and for poor flours it should be somewhat less. Whether or not the beneficial effects of this optimum hydrogen-ion concentration are due to the influence of the hydrogen ion on water imbibition by the gluten or to the securing of the proper reaction of the medium for the optimum growth of the yeast and zymase activity is a problem for further investigation. It is probable, however, that it is the latter factors which are principally influenced.

It is impossible to decide from the existing data the exact factors governing the imbibition of water by colloidal gels in acid solutions, but while the hydrogen ion undoubtedly plays an important rôle, it is equally probable that the undissociated molecule and the anion also strongly influence the degree of imbibition. In phosphoric acid the phosphate ions would be strongly adsorbed by the colloid, and such adsorption may play a great enough rôle in increasing imbibition to offset any depressing influence due to the greater concentration of the hydrogen ions.

However, there can be no doubt, after an inspection of figures 1 to 3, that there is an inherent difference in the colloidal properties of the different glutens. With lactic and acetic acids the P gluten rises more abruptly and to a higher maximum than with any of the others. The weaker glutens, especially W_2 and W_1, have a much flatter curve.

In oxalic and hydrochloric acids (fig. 5, 6) there is a much greater degree of uniformity between the different glutens, but we believe that even here certain differences may be detected, for the curves for the W_2 and W_1 glutens are again much flatter and have lower maximums than the P or C glutens.

ANTAGONISTIC ACTION OF SALTS UPON THE IMBIBITION OF WATER BY THE VARIOUS GLUTENS IN THE PRESENCE OF HYDROCHLORIC, OXALIC, LACTIC, AND ACETIC ACIDS

Tables I to IV and figures 6 to 17 show the imbibition data for the various glutens when certain salts in 0.005 molar concentration were added to the various concentrations of the acids.

In nearly every instance the addition of the salts decreases the amount of imbibition and also changes the form of the hydration curve so that a higher concentration of acid is necessary to produce maximum imbibition. There is also a noticeable difference in the behavior of the different glutens. Certain of these differences are recorded in Table X, where the acid concentrations are recorded for the various solutions of lactic and acetic acids at the maximum imbibition of the different glutens. The corresponding figures for oxalic and hydrochloric acids are not included, inasmuch as the curves for these "stronger" acids are much more nearly identical. It is of interest to note that whereas $N/5$ acid is the highest concentration of acid causing maximum imbibition in the acids alone, when salts are added there are 15 instances where maximum imbibition is not yet reached at an acid concentration of $N/2$. The behavior of the different glutens in these solutions is a strong argument for the hypothesis that the physico-chemical properties of the glutens are not identical. This is especially true for the curves for calcium chlorid, magnesium chlorid, and aluminium sulphate. As we pass from P gluten to C gluten and finally to W_3 gluten, these curves show less tendency to reach maximums and then decline. The curve for lactic acid plus calcium chlorid is particularly striking.

TABLE X.—*Concentration of the lactic and acetic acids at the maximum point on the various imbibition curves*

Solution.	Lactic acid.			Acetic acid.		
	P	C	W_2	P	C	W_2
Acid alone	*N/10*	*N/25*	*N/10*	*N/5*	*N/10*	*N/10*
Acid+potassium chlorid	*N/5*	*N/10*	*N/5*	> *N/2*	*N/10*	> *N/2*
Acid+potassium phosphate	> *N/2*	*N/5*	*N/10*	*N/5*	> *N/2*	> *N/2*
Acid+potassium tartrate	> *N/2*	*N/5*	*N/5*	> *N/2*	> *N/2*	> *N/2*
Acid+calcium chlorid	*N/5*	> *N/2*	> *N/2*	.....	.....	.....
Acid+magnesium sulphate	*N/5*	*N/5*	> *N/2*	.....	.....	.....
Acid+mercuric chlorid	< *N/500*	< *N/500*	< *N/500*	.....	.....	.....
Acid+aluminium sulphate	> *N/2*	> *N/2*	> *N/2*	.....	.....	.....

The imbibition curves in the presence of lactic acid plus mercuric chlorid are the reverse form from the curves in the presence of the other salts. Here maximum imbibition takes place in the 0.005 molar solution of mercuric chlorid, and when acid is added, the imbibition rapidly decreases. The more inferior the gluten the lower is this minimum imbibition. When, however, the lactic acid is replaced by hydrochloric acid, maximum imbibition does not take place in the 0.005 molar solution of mercuric chlorid alone, but in solutions containing both the mercuric chlorid and hydrochloric acid. The imbibition curves here are comparable in form to those for the other salts. Just what factors cause this reversal of form of the curves in the two instances is uncertain, and the subject was not further investigated, inasmuch as it was thought to be of more theoretical than of practical interest.

TABLE XI.—*Average imbibition of the different glutens in the various solutions of acids and of acids plus salts*

Solution.	Lactic acid.			Acetic acid.			Oxalic acid.			Hydrochloric acid.		
	P	C	W_2	P	C	W_2	P	C	W_2	P	C	W_2
Acid alone[1]	1.01	0.85	0.82	0.86	0.78	0.70	0.41	0.45	0.40	0.43	0.40	0.48
Acid+potassium chlorid	.69	.62	.56	.42	.53	.41	.30	.33	.31	.32	.25	.31
Acid+potassium phosphate	.64	.69	.62	.50	.49	.42	.48	.39	.39	.35	.29	.35
Acid+potassium tartrate	.65	.67	.52	.40	.46	.34	.30	.36	.30	.27	.29	.30
Acid+calcium chlorid	.52	.52	.50	.....	.....	.....	.....	.....	.....	.26	.19	.29
Acid+magnesium sulphate	.32	.27	.27	.....	.....	.....	.....	.....	.....	.15	.06	.16
Acid+mercuric chlorid	.36	.33	.15	.....	.....	.....	.....	.....	.....	.34	.28	.30
Acid+aluminium sulphate	.33	.33	.27	.....	.....	.....	.....	.....	.....	.17	.16	.17

[1] The figures on W_1 and W_2 glutens were, respectively; Lactic acid alone, 0.65 and 0.49; acetic acid alone, 0.32 and 0.48; oxalic acid alone, 0.38 and 0.39; and hydrochloric acid alone, 0.30 and 0.31.

Table XI shows the average imbibition of the different glutens in the different acids and salts. Each of these figures represents the average point of an entire curve, and as such represents from 24 to 56 individual determinations of the weight of water imbibed. The averages of such a large number of determinations certainly ought to have significance.

Here again the differences in the stronger acids do not appear very striking. Those for lactic acid are, however, very consistent. In every instance the W_3 gluten shows lower imbibition than P gluten, and the antagonistic action of salts on imbibition by the P gluten is very much more pronounced than with either C or W_3 gluten.

There is a certain similarity between the curves for the P gluten and salt and those for the weaker glutens in acid alone. There is, however, no necessary correlation, for a flat, slowly rising curve means only that the imbibing power of the gluten is low. In one instance this is due to the antagonistic action of the salts upon the action of the acid and in the other instance to an inherently weaker tendency on the part of the colloid to imbibe water. That these differences actually do exist in the two instances is shown by the fact that the presence of salts in the acid solution causes the gluten to retain its coherence and become more firm and elastic than controls in the same concentration of acid lacking the salts. This does not hold for the weak glutens with the flat imbibition curves, for these lose their coherence, become weak and inelastic and disperse at a much lower degree of hydration than do those glutens whose curves rise sharply.

We can therefore definitely state that a weak gluten does not owe its "weakness", nor its imbibition curve its "flatness", to either the acid or the salt content of the flour from which it is derived, but rather to the fact that a weak gluten has inherently inferior colloidal properties.

BAKING TESTS AND FLOUR ANALYSES

In the foregoing experiments and discussion, considerable attention has been directed to the effect of acids and salts upon glutens prepared from strong and weak flours, and we believe that it has been clearly shown that the determining factor in flour strength is not the concentration of soluble acids and salts which are present in the flour.

Further evidence, however, that "quality" in flours is not determined by the soluble-acid and salt content is again presented in Table VI. From the data therein given, it is to be observed that the patent flour ranks first in baking quality. It absorbs more water in the doughing process, producing a dough much more coherent and elastic, as is shown by the maximum expansion of the dough during the process of fermentation, and produces a loaf of the largest size and of the best texture.

It will also be noted that the patent flour is somewhat lower in its total and soluble ash content, and electrical conductivity. However, the differences between the patent flour, the flour with the strong gluten, and the W_3 flour, the flour with much weaker gluten, give values for ash on dry flour, soluble ash, and specific conductivity of the flour extract, all of which are within experimental error of each other, an observation which confirms the previously expressed idea that strength or weakness of gluten is due to the colloidal condition of the flour proteins, and is not

determined to any great extent by the inorganic material present in the flour. The soluble ash and specific conductivity are almost parallel to each other in every case. This was to be expected, since the determinations were made on identical flour extracts.

The bread baked from the weak flours was of poor texture. In each case the dough lacked the coherency and elasticity necessary to produce "large well-piled" loaves. This is without doubt partially but not entirely accounted for by the lower gluten content of these flours.

WHAT DETERMINES THE PHYSICAL STATE OF THE GLUTEN

Upson and Calvin (*14*, *15*) believe the differences between strong and weak glutens to be due to acids or salts in the flour, but it has been shown earlier in this paper that there is no evidence for such an assumption.

Wood (*17*) likewise states that the physical properties of the gluten are due—

> to varying concentrations of acid and soluble salts in the natural surroundings of the gluten.

This would appear to be identical with the view of Upson and Calvin, but while Upson and Calvin believe that the controlling factors are present in the flour, Wood (*17*, *p. 274*) believes that the character of the gluten is altered at the time when it is laid down in the wheat kernel.

> It must be decided at what stage the acids and salts influence the gluten so as to mpress upon it the physical characters which decide the physical character of the flour. I take it that this must occur when the endosperm is being formed, at which time the grain contains much more water than when it is ready to grind.

Wood and Hardy (*17*) suggest that each particle in a gluten hydrosol is surrounded by an electric double layer and that the—

> tenacity, ductility, and water-content of a solid mass of moist gluten depends upon the total or partial disappearance of these electric double layers, and the reappearance of what is otherwise obscured by them, namely, the adhesion or "idio attraction," as Graham called it, of the colloid particles for each other, which makes them cohere when they come together—the most complete coagulation, i. e., mechanically the densest and most coherent coagulum being formed at the isoelectric point.

However, in all of the papers by Wood or Hardy the assumption is apparently made that at the isoelectric point all glutens are identical in physico-chemical properties. This we do not believe to be the case, for if all glutens were identical at the isoelectric point and the degree of hydration, etc., were regulated by the presence or absence of electric double layers around the colloidal particles, we should expect to find approximately the same maximum hydration capacity for each gluten preparation, although the maximum point on the hydration curves might be reached at different concentrations of acids. We have already shown that the glutens from different sources differ in rate of hydration and in their maximum hydration capacity, and we believe that these

factors show that even at the isoelectric point there would be wide differences in their physical properties. It appears extremely probable that the actual cause of the physico-chemical differences between strong and weak glutens may be due to the form in which the protein is laid down in the endosperm. It is entirely within reason to suppose that the gluten may, under certain environmental conditions, be deposited as uniformly-sized particles with the characteristics of true emulsoids, while under different environmental conditions a part of the gluten may be deposited in this form and another part in a semicrystalloidal form. In other words, we postulate that the particles in a weak gluten are on the average nearer the boundary line which separates the crystalloidal state of matter from the colloidal state than are the particles comprising a strong gluten. It is the intention of one of us to test this hypothesis in the near future.

SUMMARY

In this paper are presented data showing the increase or decrease of water imbibition caused by immersing weighed disks of gluten from five selected flours in solutions of lactic, acetic, boric, phosphoric, hydrochloric, and oxalic acids of various concentrations, both with and without the addition of 0.005 molar concentrations of certain salts.

Data have also been presented showing different flour analyses such as ash on dry flour, soluble ash, specific conductivity of flour extract, percentage of moist gluten, percentage of dry gluten, percentage of ash in dry gluten, and baking tests.

From a study of these data, the following conclusions have been drawn:

(1) Although the moist glutens from these flours differ widely in "quality" and in physical properties, they are hydrated to almost exactly the same extent.

(2) Gluten from a weak flour has a much lower rate of hydration than gluten from a strong flour.

(3) Gluten from a weak flour has a much lower maximum hydration capacity than gluten from a strong flour, changing from a gel to a sol at a much lower degree of hydration.

(4) Two types of imbibition curves were observed. Dilute solutions of hydrochloric acid and of oxalic acid cause the gluten to rapidly imbibe water, while at slightly stronger concentrations of acid water is actually extracted from the moist gluten. Dilute solutions of lactic, acetic and phosphoric acids cause the gluten to strongly imbibe water but stronger acid solutions only slightly diminish the imbibition. The hydrogen-ion concentration of the acid is not the only factor influencing imbibition, but it is pointed out that the anion and the undissociated molecules, as well as their relative adsorption by the protein, must in all probability be taken into consideration.

(5) Inorganic salts when added to an acid solution lower the relative imbition of gluten placed in such solutions. Glutens from the different flours react differently to the addition of inorganic salts.

(6) The acid and salt contents of the flours are not responsible for the difference between a strong and weak gluten.

(7) The postulation that the different physical conditions observed in glutens derived from different flours are due solely to the presence or absence of an electric double layer around the colloidal particles is not consistent with the facts recorded in this paper. A strong gluten would differ from a weak gluten even at the isoelectric point.

(8) There is an inherent difference in the glutens from the strong and weak flours. The physico-chemical properties of the glutens from the different flours are not identical and would not be identical even if the flours had originally had the same acid and salt content.

(9) The difference between a strong and weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which these properties are much less marked. It is suggested that such differences may be due to the size of the gluten particles and that at least a part of the particles comprising the weak gluten may lie nearer the boundary between the colloidal and crystalloidal states of matter than is the case with the stronger glutens.

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CHEMISTRY AND HISTOLOGY OF THE GLANDS OF THE COTTON PLANT, WITH NOTES ON THE OCCURRENCE OF SIMILAR GLANDS IN RELATED PLANTS[1]

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INTRODUCTION

The work herein reported forms a portion of a chemical and biological investigation of the cotton plant (*Gossypium* spp.), the purpose of which is to isolate and determine the substance or substances which attract the boll weevil. A previous paper (*17*)[2] discusses the isolation of certain glucosids and the products of their hydrolysis, as well as preliminary studies of an ethereal oil which manifested some attraction for the boll weevil. Both the glucosids and this oil, as well as several other substances, are largely localized in prominent internal glands which are very numerous in nearly all parts of the cotton plant. The main purpose of this paper is to discuss the occurrence, formation, structure, and contents of these glands.

Glands of another type, more properly referred to as "nectaries," also occur in the cotton plant. These are superficial in position and definitely localized. The internal glands have nothing in common with these nectaries save the function of secretion. In certain taxonomic and other literature, however, either or both types are referred to indiscriminately simply as "glands." Therefore, it seems advisable also to discuss briefly in this paper the nature and occurrence of the nectaries, in order to distinguish them clearly from the internal secretory organs, which form the main subject of the present study.

I.—THE INTERNAL GLANDS

The internal gland of cotton consists of an oblate or spheroidal central sac 100 to 300 μ in diameter, filled with a more or less homogenous yellow or brownish secretion, surrounded by an envelope of one or more layers of flattened cells, which in the glands exposed to light contain a red pigment.

DISTRIBUTION OF INTERNAL GLANDS

Because of their dark color, which renders them plainly evident beneath the epidermis of the green stem or palisade layer of the foliage, and because of the supposed nature of their content, the internal glands have been

[1] Second paper of a series on the chemistry of the cotton plant, with special reference to Upland cotton.

[2] Reference is made by number (italic) to "Literature cited," p. 434-435.

variously alluded to as "black glands," "gland-dots," "resin glands," "oil glands," "gossypol glands," etc. They are constantly present and definitely arranged throughout the genus Gossypium, although their prominence varies in different species according to their size, proximity to the surface, depth of pigmentation, and presence or absence of obscuring hairs or tomentum.

Within the seed (Pl. 42, A, C) the glands are found directly beneath the palisade layer, into which they often project, causing a shortening of the palisade cells, but usually no bulging of the surface. Here they are oblate-spheroidal in form, with long axis perpendicular to the cotyledon surfaces. Their long axes frequently exceed half the width of the cotyledon—that is, 100 to 200 μ. Smaller glands are also found in the cortex of the radicle, covered by a few parenchymal layers.

As the seedling develops, glands are formed profusely in the primary cortex of the hypocotyl (Pl. 43, A, B; 44, A, B) and sparingly in that of the radicle (Pl. 45, A, B). In the former they are nearly globular, and in the latter much elongated. In the hypocotyl and the young stem the glands occur very close to the epidermis, and often push it outward in their development, appearing then like small, dark warts. Their formation keeps pace with the development of the foliage; in the unfolded cotyledons and true leaves they are located beneath the palisade layer in the centers of small areas bounded by the anastomosing veins. Stipules, bracteoles, and also the calyx and corolla (Pl. 46, A, *a*, *b*) are glandulate, the arrangement of glands being in general similar to that of the foliage, though not all netted areas possess glands. Glands occur also within the tissues of the anther and the staminal column (Pl. 46, A, *c*, *d*). The principal veins of the bracteoles frequently appear nonglandulate, in marked contradistinction to the areas between them; on inspection with a hand lens, however, small glands may usually be seen upon them. The veins of the calyx are nearly destitute of glands; these veins are set closely together, and the glands between them therefore seem to run in parallel lines. Glands occur plentifully upon the style, in rows between and below the stigmas. The boll possesses relatively very large glands; in *G. hirsutum* L. (Pl. 46, B) they are beneath several cell layers, and are accordingly less conspicuous than in *G. barbadense* L., where they are close to the surface, which is pitted above them. The glands of the green parts are very nearly spherical in form.

The secondary cortex also may contain glands of a slightly different type (Pl. 47, A). These always occur within the expanded ends of the medullary rays. The formation of glands in the secondary cortex would seem to be influenced by the stimulus of light, They are rarely formed in the secondary cortex of the stem (Pl. 47, B) and here apparently only where the outer tissues have become considerably suberized and opaque. They occur, however, very plentifully in the root; several are usually found in each phloem ray.

No glands are found in the xylem or phloem proper nor in the seed coats.

DEVELOPMENT OF INTERNAL GLANDS

Internal glands, with the exception of those of the secondary cortex, are developed from certain cells of the ground meristem. Those of the seed are formed coincidentally with the development of the tissues of the embryo, which are formed from the endosperm soon after fertilization takes place. The process of gland development in all parts of the plant, except the secondary cortex, is essentially the same. Within the rapidly dividing tissue of the ground meristem a well-defined circle of cells marks the boundary of the developing gland; the cells within this circle are more or less concentrically placed (Pl. 48, A). A marked change of content takes place in the central cells; the protoplasm becomes vacuolate and arranged in strands and is converted into a yellowish oily-appearing substance (Pl. 48, B, C). These changes are accompanied by rapid swelling of the cells concerned, some of which are crushed and obliterated in the process, while the peripheral layers become much flattened. The nuclei of the swelling cells enlarge and soon degenerate and disappear. The walls of the swollen cells usually are dissolved and disappear rapidly, leaving the gland as a large central cavity surrounded by layers of the flattened cells. In the seed glands (Pl. 42, C), however, the interior walls never wholly disappear, but vestiges, easily dissolved in water and probably of a mucilaginous nature, remain. The writers regard the process of gland formation as truly lysigenous, for traces of the secretion can be observed in the unbroken cells, and not, as the more common schizogenous glands, first in the intercellular spaces, as figured by Tschirch (*15, p. 1095–1268*).

While the secretion is first formed in the central cells, its general solidification in the seed indicates that it may be added by the flattened cells in the encircling layers, which then in their turn may act as secretory cells, as in a schizogenous gland. The presence of this layer, usually characteristic of the latter type of gland, is doubtless the cause for Dumont's (*2*) reference to the glands of *Gossypium* spp. as schizogenous. The cells of the encircling layers retain their nuclei and their walls at length become considerably thickened. In the seed they are somewhat mucilaginized, dissolving partially in water and cuprammonia, but not in alcohol, and giving no well-defined cellulose reactions. In this latter respect, however, they do not differ from the surrounding cell walls, which are not water-soluble.

The development of glands in the secondary cortex is similar, but usually simpler. One or more cells may be involved in the process. Upon their division from the cambium the nuclei quickly disappear, the cells become filled with a dense yellowish oily substance, and enlarge rapidly. When more than one cell is involved, the dissolution of the dividing walls is more rapid than in the glands previously described. The distension of the secretory cells flattens one or two surrounding cell layers into an envelope resembling that of the primary gland.

SECRETIONS OF THE INTERNAL GLANDS

Microscopical investigations of the secretion of the internal gland have shown the presence of a variety of substances and also of differences of content, according as the glands are or are not exposed to the action of light. Most evident are a deep-red pigment and a yellow or brownish oily-appearing substance. The red, or sometimes purple, pigment is deposited in amorphous semisolid or in liquid form within the flattened layers of the glands of illuminated parts. It is most prominent in the glands of the young green parts, and its density accounts for their "black" appearance; the red coloration may be made microscopically evident by crushing the gland, thereby diluting the pigment in cell sap. The coloring of conspicuous red spots on cotton foliage often originates in wounded glands. No red pigment is normally formed in the glands of the seed or secondary cortex. A small amount is usually developed in the glands of the cotyledon when the latter becomes functional as a leaf. Pigment is first formed in the glands of the petals as the latter protrude from the bud.

This red pigment gives the reaction typical of anthocyans. It is soluble in water and alcohol, nearly insoluble in ether, and insoluble in petroleum ether. Acids dissolve it with a brilliant red coloration. Green or blue colors are first formed with alkalis; strongly alkaline solutions are soon decolorized. Basic lead acetate forms a dark-green precipitate. Iron salts cause a blue or purple coloration. The red pigment of the glands is apparently more strongly developed in *G. barbadense* than in *G. hirsutum*. If a young leaf of the former species be slightly crushed, the diffusion of anthocyan may color the whole injured area a brilliant red.

The content of the central chamber of the gland varies from a yellow oily fluid in the young gland to a resinous-red solid substance in the mature gland of the seed. With proper reagents the yellow secretions of the glands exposed to light are seen to differ in character from those of the inner cortex of the stem, root bark, seeds, and the partially developed corolla, which, by their positions, are shielded from the influence of light.

SECRETIONS OF THE GLANDS EXPOSED TO LIGHT

In the large glands of the boll occurs a bright-yellow oily substance in which may be intermixed granular fragments of a dull-yellow or orange color. In the smaller glands of other outer portions of the plant the yellow substance occurs in smaller amount; the solid sometimes predominates. Reactions show the solid, in the main, to be a condensation product of the yellow liquid. The liquid, which flows out in globules as the gland is cut, is nearly insoluble in water and is not readily emulsified in it. It is very soluble in ethyl and methyl alcohol, acetone, chloroform, and ether, forming a bright-yellow solution. It is almost or quite insoluble in petroleum ether and xylene. In most acids

it is wholly or nearly insoluble. In alkalis the globules swell, become orange in color, and dissolve, forming a bright-orange solution which becomes yellow on dilution. A dark-green precipitate is formed by alcoholic ferric chlorid, and an orange or yellow precipitate by lead acetate. These reactions are characteristic of flavones. Quercetin and several of its glucosids have been shown by Perkin (*9*, *10*, *11*) to be present in the cotton plant, and a previous paper (*17*) from this Bureau discusses the isolation of quercetin and two of the glucosids, quercimeritrin and isoquercetin, from the flowers and foliage of the species here considered. The latter glucosid was present only in very small amount. The reactions of these substances correspond well with those of the yellow globules of the glands, and no reactions of flavones have been noted in other portions of the green parts.

SECRETIONS OF GLANDS NOT EXPOSED TO LIGHT

The secretion of the young glands which are not exposed to light also consist of a yellow fluid, with a slightly greenish tinge. In a mature or dried gland it becomes hardened into a reddish-resinous solid. It differs from the secretion of the glands mentioned in the preceding paragraph in forming a greenish emulsion with water and aqueous reagents. It is soluble in alcohol, ether, acetone, and chloroform, but is insoluble in petroleum ether and xylene. Its behavior with alkalis is similar to that of the secretion of the illuminated glands. The chief reaction by which it may be identified within the gland is an intense red coloration induced by concentrated sulphuric acid, which reagent dissolves quercetin and its glucosids with more or less difficulty, forming a yellow solution. This red reaction, first noted by Hanausek (*5*) was cited by Marchlewski (*7*) as a property of gossypol, which Withers and Carruth (*19*) consider to be the toxic agent in cotton seed. This red reaction is characteristic of all stages of the glands of the seed and secondary cortex, but is absent in those of the primary cortex and foliage. Glands of the developing petal at first give the gossypol-red reaction, but before or soon after the petal unfolds they lose this property and react as the glands of the green parts. Synchronously with this change of character occurs the development of anthocyan in the enveloping cell layers. A strip of petal may show all gradations from intense red to yellow reactions with sulphuric acid, according as the glands have been exposed to the light. These changes were clearly marked in blossoms of *G. barbadense*, and were confirmed in field-grown specimens of *G. hirsutum*. No gossypol was recovered from 800 gm. of ground, dried cotton flowers by the method by which Withers and Carruth (*19*) isolate the substance from cottonseed kernels. Their process consists in precipitating gossypol from the ether extract by petroleum ether.

Glands of blossoms of the Upland cotton plant which had been grown in a greenhouse whose roof was so heavily painted as to prevent largely

the passage of light, never lost their gossypol reaction; moreover, little or no anthocyan was found, either in the glands or the petals.

With the unfolding of the cotyledon in the seedling the preformed gossypol undergoes a change; it gives a brownish or greenish emulsion or solution with sulphuric acid. The anthocyan pigments give a similar but less intense red with sulphuric acid; they, moreover, react with acid in any strength, while gossypol develops the red coloration only with concentrations of 80 per cent (volumetric) or more, and the coloration is immediately lost if the gossypol-red solution be diluted below this strength.

Table I, which gives the microchemical reactions of the contents of the gland chambers of parts exposed and not exposed to light, is herewith appended. The more important reactions are compared with those of pure quercetin and quercimeritrin, prepared in this Bureau, and pure gossypol, kindly furnished by Dr. F. E. Carruth, of the North Carolina Experiment Station. Experiments to distinguish quercetin from its glucosids *in situ* have thus far not been successful.

TABLE I.—*Microchemical reactions of the secretions of the internal glands of cotton and of the substances isolated therefrom*

Reagent.	Glands unexposed to light.	Glands exposed to light.	Gossypol.	Quercimeritrin.	Quercetin.
None	Greenish-yellow fluid to reddish-brown solid.	Bright-yellow globules and slightly darker solid.	Yellow crystals	Yellow crystals	Yellow crystals.
Distilled water	Rapidly emulsified, yellow, or olive-green.	Emulsified slightly or not at all.	Insoluble	Insoluble	Insoluble.
Alcohol, absolute	Slowly dissolving yellow	Rapidly dissolving; yellow	Soluble; yellow	Slowly soluble; yellow	
Alcohol, 95 per cent	do	Rather slowly dissolving; yellow.	do	do	
Alcohol, 70 per cent	Very slowly dissolving; yellow.	Very slowly dissolving; yellow.	Slowly soluble; yellow	Very slowly soluble; yellow	
Alcohol, 50 per cent	Slowly emulsified and slightly dissolving.	Dissolving little or not at all			
Methyl alcohol	Rather rapidly dissolving	Rapidly dissolving			
Amyl alcohol	Slowly dissolving	Rather rapidly dissolving	Readily soluble		
Ether	do	Rapidly dissolving	Rapidly soluble	Nearly or quite insoluble	Nearly or quite insoluble.
Chloroform	do	do	do		
Acetone	Rather rapidly dissolving	Instantly dissolving	do	Rapidly soluble	
Acetic ether	Slowly dissolving	Rapidly dissolving	do		
Petroleum ether	Not dissolving	Not dissolving	Not soluble	Not soluble	
Xylene	do	do	do	do	
Carbon disulphid	Rather rapidly dissolving	Rather rapidly dissolving	Readily soluble		Slowly soluble.
Carbon tetrachlorid	Little or not at all dissolving	do	do		Do.
Anilin	Very rapidly dissolving; bright yellow.	Rapidly dissolving; orange to yellow.	Immediately soluble; bright yellow.	Slowly soluble	
Glycerin	Very slowly dissolving	Slowly dissolving	Insoluble cold; soluble hot		
Chloral hydrate	Emulsified, slowly dissolving; greenish yellow.	Emulsified, slowly dissolving; yellow.	Slightly soluble	Slowly soluble	
Cottonseed oil	Dissolving on heating	Dissolving at about 50° C.	Very slowly cold; readily at 60° C.		
Volatile oil distilled from cotton plants.	Slowly dissolving cold; less slowly with heat. Practically all dissolved at 70° C. in 5 minutes (seed).	Readily dissolving in cold. Anthocyans dissolve with heat at 50° to 60° C.	Readily soluble in cold; greenish yellow.	Readily soluble in cold. Yellow.	Slowly soluble in cold.
Acid, acetic (concentrated)	Slowly dissolving; yellow	Quite rapidly dissolving	Very slowly soluble	Slightly soluble	Slightly soluble.
Acid, hydrochloric (concentrated).	Emulsified, olive or yellow-green.	Not dissolving	Insoluble	Slowly soluble (?)	
Acid, nitric (concentrated)	Slowly dissolving; orange	do	Nearly insoluble		
Acid, sulphuric (concentrated).	Immediatly dissolving; brilliant red.	Dissolving; yellow to orange	Immediately soluble; brilliant red.	Rapidly soluble; bright yellow.	Rapidly soluble; yellow to orange.
Acid, sulphuric (concentrated), 80 per cent.	Rapidly dissolving; brilliant red.	Slowly, or not at all, dissolving; yellow to orange.	do		
Acid, sulphuric (concentrated), 65 per cent.	Emulsified; brown	Not dissolving	Slightly soluble; pink		
Acid, sulphuric (concentrated), 50 per cent.	do	do	Insoluble, hot or cold		

TABLE I.—*Microchemical reactions of the secretions of the internal glands of cotton and of the substances isolated therefrom*—Continued

Reagent.	Glands unexposed to light.	Glands exposed to light.	Gossypol.	Quercimeritrin.	Quercetin.
Sulphuric acid plus absolute alcohol, equal parts.	Immediately dissolving; brilliant red.	Scarcely dissolving	Rapidly soluble; brilliant red.		
Ammonium hydroxid (concentrated).	Emulsified, dissolving, bright yellow.	Globules coalescing, slowly dissolving; orange, brown to yellow.	Rapidly soluble; yellow	Immediately soluble; orange to yellow.	Immediately soluble; orange to yellow.
Potassium hydroxid, 10 per cent.	Rapidly dissolving; bright yellow.	Globules rather rapidly dissolving; orange to yellow.	do	do	Do.
Potassium hydroxid, 10 per cent alcohol.	Immediately dissolving; bright yellow, becoming purple on heating.	Immediately dissolving; bright orange, becoming yellow on dilution.	Rapidly soluble; becoming purple on heating; yellow on standing.	More slowly soluble; orange to yellow.	More slowly soluble; orange to yellow.
Eau de Javelle	Swelled, emulsified, olive-green to yellowish.	Dissolving; yellow	Scarcely soluble		
Phloroglucin alcohol	Slowly dissolving; yellow	Slowly dissolving			
Phloroglucin alcohol plus hydrochloric acid.	No change	No change			
Vanillin plus hydrochloric acid.	Emulsified, dissolving; yellow; pink reaction appears slowly, at length very pronounced. Reaction appears in cortical tissues of root before it is seen in the glands.	Slowly dissolving; yellow, pink reaction in surrounding tissues; not in glands.			No reaction.
Alkannin	Darker, becoming reddened	Bright red, slow			
Sudan III in 70 per cent alcohol.	Dissolving without much color change.	Bright red, rapid; globules dissolving.			
Acid, osmic, 1 per cent	Secretion darkens, becoming black.	Blackened			
Potassium dichromate, 10 per cent.	Emulsified, brown particles not dissolving.	Partially precipitated; yellow to brown.			
Chromo-acetic fixative, 24 hours.	Solidified, not dissolving in alcohol; sulphuric red reaction fails or develops only slowly and partially.	Partially solidified; not dissolving in alcohol.			
Ferric chlorid, 10 per cent alcoholic.	Darkened; not dissolving	Dark green precipitate			
Ferrous sulphate, 10 per cent	Darkened; greenish	No color change			
Lead acetate	Precipitation from solution without much color change.	Precipitation without much color change.		Precipitated from solution; yellow.	Precipitated from solution; orange to yellow.
Cuprammonia	Darkened, emulsified, enveloping cells somewhat dissolving.	Scarcely dissolving; darkened, enveloping cells dissolved.			
Chlorzinciodin	Slowly emulsified; no cellulose reaction in enveloping cells of seed.	Cellulose reaction in enveloping layer.	Insoluble		
Iodin plus sulphuric acid	do	do			

Ammonium molybdate	Not much change of color in alkaline solution, with or without ammonium chlorid.	Orange-red in alkaline solution.	Not orange-red in alkaline solution.	Orange-red in alkaline solution.
Copper tartrate in sodium hydroxid, warmed.	Reaction lacking or doubtful.	Precipitate of copper oxid in varying degrees.		
Alpha-napthol in sulphuric acid.	Dissolving; bright green	Purple	Soluble; bright green	Soluble, bright yellow; becoming greenish in contact with air.

Some discrepancies, especially in the matter of solubility, may readily be noted between the gland secretions and the pure substances which, the writers believe, make up their principal constituents. Two explanations, either or both of which may be correct, may be cited in respect to this point:

(1) Quercimeritrin and gossypol may be present in the form of salts whose solubilities differ from those of the pure substances. Perkin (*10*) believes quercimeritrin to exist in the petals of *G. herbaceum* in the form of a potassium salt. In proof of this view he cites (a) the ready solubility in water of the crude dyestuff from the corollas, in contrast to the relative insolubility of quercimeritrin; (b) the presence of a large quantity of potassium in the ash of his crude water extract; (c) the preparation from quercimeritrin of a monopotassium salt readily soluble in water. With respect to *G. hirsutum*, however, the writers do not find the gland secretion to be appreciably soluble in water (though this may be due to the protective action of an oil in which the flavone is dissolved. This will be again referred to). Also, as previously described (*17*) pure quercimeritrin has been prepared in this bureau directly by crystallization from an alcohol extract of corollas of *G. hirsutum*, without the treatment with lead acetate which Perkin employed with the species he investigated.

(2) The discrepancies in question may be due to the presence in the gland of other substances, especially of an ethereal oil in which the dyestuffs are dissolved. This oil would protect the dyestuffs against the action of reagents immiscible with it in which the solutes are less soluble (aqueous reagents). Conversely, the oil would render the dyestuffs apparently more soluble in reagents which mix readily with it—for example, ether—yet in which pure substances (quercimeritrin in the case of ether) are almost or quite insoluble. While no microchemical reaction has been developed which will definitely distinguish between quercetin and its glucosids, previous work (*17*) showed the presence in the corollas of a comparatively large amount of quercemeritrin, while the foliage yielded much quercetin and little quercimeritrin. It seems probable, therefore, that the glands of the corolla contain principally quercetin. This latter conjecture is strengthened by the sugar reaction given by the glands of the green parts, which indicates a probable enzymic hydrolysis.

SECRETIONS MORE OR LESS COMMON TO BOTH TYPES OF INTERNAL GLANDS

The presence of oil as a solvent of the flavone substances is indicated by the appearance of the globules and their ready coloration with alkannin, Sudan III, and osmic acid confirms this conjecture. The globules are reduced to solid form by treatment with dry heat at 100° C. or by steam; this and their ready solubility in alcohol indicate that the oil is of a volatile nature. A volatile oil whose properties are now under investigation has been prepared by the steam distillation of fresh cotton plants.

Other substances doubtless occur within the internal glands. A resin reaction is obtained by prolonged treatment with copper acetate. Prolonged treatment with chromic-acid reagents results in the conversion of apparently the whole content of the glands not exposed to light into a resinous-brown solid which is insoluble in water and alcohol and which gives the sulphuric-acid red reaction slowly, or not at all. The contents of those exposed to light form a yellow or dark-brown precipitate upon the walls of the cavity. This property of precipitation with chromic acid indicates that some of the contents of the glands may be closely allied to tannin.

Vanillin-hydrochloric acid gives, after several minutes, a strong phloroglucin reaction within the glands of the seed and root. None has been noted within the glands of the other parts, but phloroglucin is present within the adjoining cells. Sugar is frequently present in the glands exposed to light. Positive tests are given with α-napthol, sulphuric acid, and alkaline copper tartrate. The presence of sugar is somewhat doubtful in the other glands.

PRESENCE OF FLAVONE SUBSTANCES OUTSIDE GLANDS

In the green parts flavones are found only in the gland secretion. In the roots they occur in the outside cortical layers. They give the gossypol-red reaction and also a strong precipitation with tannin reagents. Pollen grains are colored bright red by sulphuric acid; those of *Malva silvestris* L. show a similar reaction. Flavones which agree with those of the green parts in forming a yellow solution with sulphuric acid occur in the seed, largely in the palisade layers of the cotyledons. Similar substances give the yellow coloration to the petals of *G. barbadense*, and are found to a less extent in the unfolding, nearly colorless or yellow corollas of *G. hirsutum*, being later obscured by anthocyans; but they are still capable of being shown by reagents. It is probable that the flavones in the glands form but a small proportion of the total amount present in the corolla.

RELATIONSHIP BETWEEN GOSSYPOL AND QUERCETIN AND ITS GLUCOSIDS

Marchlewski (7) terms gossypol a "dihydroxy phenolic substance," and proposes for it the formula $C_{13}H_{14}O_4$, with $C_{32}H_{34}O_{10}$ as an alternate formula. Withers and Carruth (*19*) consider that Marchlewski's substance contained acetic acid in combination. Their paper, which discusses the physiological effect of gossypol, does not deal extensively with the chemistry of the substance. They are now preparing publications which, it is to be hoped, will clear up the uncertainty which at present exists regarding the composition of gossypol. In the present state of our knowledge the exact chemical relation of gossypol to quercetin and its glucosids is uncertain. Perkin (*9*), referring to the work of Marchlewski on gossypol, states:

> It [gossypol] . . . does not appear to be closely allied with that (dye) present in the flowers.

This was before his discovery of quercimeritrin. Gossypol is evidently a somewhat unstable substance; Withers and Carruth (*19*) state that it readily forms an oxidation product which is physiologically inert. Its nature is also evidently changed by moist heat. Osborne and Mendel (*8*) find boiled cottonseed kernels to be nontoxic. The investigations of the present writers show that it is not formed, or if formed as a preliminary step in the synthesis of the quercetin glucosids, is immediately changed in those tissues which are exposed to the action of normal daylight. As gossypol occurs in the glands of the flower before opening and quercimeritrin after opening, the former apparently in this case gives rise to the latter; but the exact nature of the change remains uncertain. This change of gossypol differs from that in the unfolding of the cotyledons. Here no quercimeritrin seems to be produced, the gland content giving a greenish-brown emulsion with sulphuric acid.

RELATIONSHIP BETWEEN THE FLAVONES AND THE ANTHOCYANS

A definite relationship between the flavone glucosids and the anthocyans is assumed by Perkin (*11*) in citing the experiments of Everest (*3*), who formed anthocyans by a reduction of certain glucosids. The constant association of the two in the glands exposed to light, their immediate formation in those of the corolla as the petals unfold, and in the full-blown flower, the development of anthocyans in cells previously containing flavones and still containing them, point to a photochemical process resulting in the development of anthocyans from the glucosid. It is not asserted, however, that the development of anthocyan is here necessarily dependent on the preformation of glucosid in demonstrable quantities; anthocyan is frequently developed in the epidermal cells of stems, petioles, and leaves under extreme insolation where no flavone can be demonstrated. The stimulus of light, to which the development of anthocyan in normal tissue is here conjecturally referred, seems not to be invariably necessary. In the interior of certain bolls what appeared to be an abortive second boll developed, and in the outer tissues of this were developed glands analogous to those of normal tissue, containing no gossypol, but with pronounced anthocyan-bearing envelopes, although the stimulus of light was insufficient for the development of chlorophyll in the surrounding tissues.

POSSIBLE BIOLOGICAL SIGNIFICANCE OF THE INTERNAL GLANDS

Whether or not the substances found in the glands are of definite use to the plant is an unsettled question. Many investigators, notably Haberlandt (*4 p. 526*) have regarded the contents of glands of this general type as excretion products useless to the plant, basing this conclusion largely on the fact that they remain thus localized indefinitely without change. The change of gossypol upon the unfolding of the cotyledon may indicate its usefulnesss in the metabolism of the young seedling.

Furthermore, its pronounced toxicity may perhaps be regarded as a protective adaptation of the seed against animal attack. Haberlandt (*4*) states that—

> excretory substances (in reservoirs of this type) are frequently made use of for protection against animal foes.

Withers and Carruth (*19*) found rabbits much averse to cottonseed kernels as a food, especially after once having been made sick. The present writers have found no suggestion of such toxicity in quercetin or its glucosids, but have had no opportunity to test them biologically. Cook (*1*) credits the gland secretion with a repellent effect on the boll worm, and discusses its possible value in repelling the boll weevil. He finds that the relatively immune Kekchi cotton is usually punctured only in areas free from glands, but states also that glands are especially well developed in Mit Afifi and Egyptian cotton varieties particularly favored by the weevil. Boll weevils which have been watched while puncturing young plants seem to avoid the glands.

Preliminary experiments were made to test the attraction for the weevil of substances derived from the cotton plant. Of these the glucosids quercimeritrin, isoquerceretin, and especially the steam distillate and a volatile oil extracted therefrom appeared somewhat attractive. These substances are largely localized in the interior glands. It is possible, in view of the fact that the boll weevil has not been seen to puncture the glands, that this attraction may consist in an odor which suggests the presence of the cotton plant rather than in any actual food value of the substances. The flavone substances possess no odor perceptible to human senses; the volatile oil, on the contrary, has a pronounced and characteristic odor.

That the secretion of the glands is not repellent to all insects is shown by the habit of certain aphids (*Aphis gossypii* Glov.) in frequently puncturing the glands of the mature leaves and in withdrawing part of their substance (Pl. 49, A). The flavones present seem not to be appreciably diminished, and it is probable that the substance withdrawn by the aphids consists largely of sugar (dextrose), traces of which can be microchemically shown. This sugar may very likely be formed by the hydrolysis of glucosids. The indifference of the boll weevil to sweet substances has been previously noted by Hunter and Hinds (*6*).

UNIVERSAL PRESENCE OF INTERNAL GLANDS WITHIN THE GENUS GOSSYPIUM

Watt (*18*) makes the following statement:

> They [the glands] are, moreover, nearly universally present, though in some species they are often obscured by the tomentum.

He also refers to them in those of his specific descriptions which enter into such comparatively minor details. A considerable number of species of Gossypium in the National and Economic Herbariums have been exam-

ined, and in no case was there any notable variation in the presence and distribution of "black glands," although there was a great difference (often as great within species as between them) in their size and prominence. None of these specimens possessed roots. The names of the species examined follow:

Gossypium arboreum L.; *G. barbadense* L.; *G. brasiliense* Macf.; *G. herbaceum* L; *G. hirsutum* L.; *G. mexicanum* Tod.; *G. nanking* Meyen; *G. microcarpum* Tod.; *G. obtusifolium wightiana* Watt; *G. palmeri* Watt; *G. peruvianum* Cav.; *G. religiosum* Roxb.; *G. schottii* Watt; *G. tomentosum* Nutt.; *G. neglectum* Tod.; *G. wightianum* Tod.: all from the Economic Herbarium, Bureau of Plant Industry, and classified according to Watt (*18*). *G. drynarioides* Seeman; *G. harknessii* Brandg.; *G. davidsonii* Kellogg: at the Natioanl Herbarium.

That the chemical nature of the contents of the glands of these various species is identical by no means follows; Perkin (*9*, *11*) has demonstrated marked distinctions in the flavone content of the flowers of *G. neglectum*, *G. arboreum*, and *G. sanguineum*.

PRESENCE OF INTERNAL GLANDS IN GENERA CLOSELY RELATED TO GOSSYPIUM

Internal glands, such as are here described, have been noted within the Malvaceae only in certain genera of the subfamily Hibisceae. Senra, Lagunaria, Hibiscus, Abelmoschus, Kosteletzkia, and Dicellostyles appear not to possess glands of this type. Thespesia, Cienfuegosia (Fugosia), Erioxylon, and Ingenhouzia (Thurberia) are all more or less glandulate. The arrangement of glands in Arizona wild cotton (*Ingenhouzia triloba* Moç, and Sesse; syn. *Thurberia thespesiodes* A. Gray) is identical with that of *Gossypium* spp., and the glands of the seed (Pl. 42, B) react like those of that genus. Only a fragmentary specimen, consisting of stem, leaf, and flower of *Erioxylon aridum* Rose and Standley, has been seen by the present writers; on this the gland arrangement was also like that in *Gossypium* spp.

Four specimens of *Thespesia* spp. (*T. lampas* D. and E.; *T. populnea* Soland; *T. macrophylla* Blume and *T. grandiflora* D. C.) showed similar glands, but their arrangement was much less definite, and their presence (in herbarium specimens) sometimes could only be demonstrated by reagents. In contradistinction to the description of Thespesia by Schumann (*13*).

Kelch nicht punktiert,

the calyx of the two first species showed well-defined though inconspicuous glands. Seeds of both were densely glandulate, and the glands gave the gossypol-red reaction. Cienfuegosia is differentiated by Schumann (*13*) in part thus:

Kelch schwarz punktiert * * * Kotyledonen nicht punktiert.

Seven specimens were available for examination; only two specimens, *C.* (*Fugosia*) *drummondii* Lewtonand *C. phlomidifolia* Garcke, possessed

seeds, and those of the last named were nonglandulate. In *C. drummondii* very inconspicuous glands were present, distributed as in *Gossypium* spp.; on *C. phlomidifolia* (a poorly preserved specimen) they were not found on the leaves. The latter specimen only of those examined possessed glands on the petals. *C. australis* Schum., *C. hakeaefolia* Hochr., *C. argentina* Gürke, *C. hildebrandtii* Garcke, and *C. heterophylla* Garcke were also examined. In all but the second inconspicuous glands were present in most parts except the petals; usually they were most conspicuous on the involucre.

Dumont (*2*) cites the presence of "*poches schizogenes*" in species of Fugosia and Thespesia as in *Gossypium* spp.

II.—THE NECTARIES

Four sets of nectaries occur in the cotton plant, one set being floral and three extrafloral. The presence, shape, and number of the extrafloral nectaries vary in different species of Gossypium, and the taxonomic value of these variations has been discussed by Tyler (*16*).

The secretory mechanism of each nectary consists of a dense aggregation of pluriseptate glandular hairs, trichomes, or papillæ, among which simple nonsecretory hairs may be scattered. The development of the papillæ of the floral nectary from modified epidermal cells has been figured and described by Reed (*12*); and in accordance with the generally accepted origin of such structures, a similar development may be inferred for those of the two other sets. At certain periods these papillæ secrete a sweet fluid which attracts bees, moths, aphids, ants, and similar insects. Its saccharine nature is evident to the taste, and it yields the reaction characteristic of sugars with Molisch's, Meyer's, Flückiger's, and Fehling's reagents. There is no evidence to show that it is attractive in the least to the boll weevil, though its usefulness in attracting insects which prey upon this pest has been cited by Cook (*1*).

The comparatively long and narrow papillæ of the floral nectary line the inside of the base of the calyx in a band 1 or 2 mm. wide. In *G. hirsutum* and closely related species the nectary is guarded from the smaller insects by a band of hairs on the calyx above it. One set, usually consisting of three extrafloral nectaries within the bracteoles and alternating with them, occurs on the outer base of the calyx. These nectaries are irregularly triangular in shape and not deeply sunken. A set somewhat similar but more decidedly sagittate, is found deeply indented in the broadened apex of the peduncle, opposite the centers of the bracteoles, thus alternating with the intrainvolucral nectaries. Trelease (*14*), speaking of *G. hirsutum*, states that the nectaries of the outer calyx and peduncle are not present on the first flowers; on subsequent blooms those of the peduncle are formed, and on still later flowers both sets occur. In a variety of *G. barbadense* examined here the inner set appeared first, the outer set being absent on early flowers or represented by one nectary only.

A third form of extrafloral nectary (Pl. 50, A, B) is found on the underside of the principal foliar vein, not far from its base. These nectaries are shallow pits, varying considerably in shape and outline. On the cotyledon no pit occurs; the nectary is represented by a small group of poorly developed, nearly nonfunctional papillæ on the lower surface of the midvein (Pl. 49, B). On the leaves of *G. barbadense* each of the three principal veins usually possesses a nectary; that of the midvein is largest and usually sagittate in outline, with the point toward the apex of the leaf.

Internal glands are frequently found in the parenchymal tissues close to the nectaries (Pl. 49, B; 50, A, B), especially those of the leaf; but they have no organic connection with them.

Septate papillæ similar to those of the nectaries occur frequently on the young green parts of the plant.

SUMMARY

(1) Internal glands of lysigenous formation are found in the primary cortex, foliage, flower, and seed of Upland cotton (*Gossypium hirsutum*).

(2) The secondary cortex contains glands of a similar type. Some of these appear to be developed from the enlargement of a single cell.

(3) The glands in portions of the plant which are exposed to light are surrounded by an anthocyan-bearing envelope of flattened cells, and contain quercetin, probably partly or wholly in the form of its glucosids quercimeritrin or isoquercitrin, ethereal oil, resins, and perhaps tannins.

(4) The glands not normally exposed to the light are surrounded by a layer of flattened cells containing no anthocyans; they contain gossypol.

(5) Gossypol is formed in the glands of the developing corolla; on their exposure to light it is replaced by quercimeritrin.

(6) Gossypol in the unfolding cotyledons is changed, probably through oxidation, without the formation of quercimeritrin.

(7) Internal glands of the type described are universally present within *Gossypium* spp.

(8) Internal glands occur to some extent within the related genera, Thespesia, Cienfuegosia, Erioxylon, and Ingenhouzia.

(9) Four types of glands which function as nectaries occur in *G. hirsutum*. These differ morphologically from the internal glands and have no connection with them.

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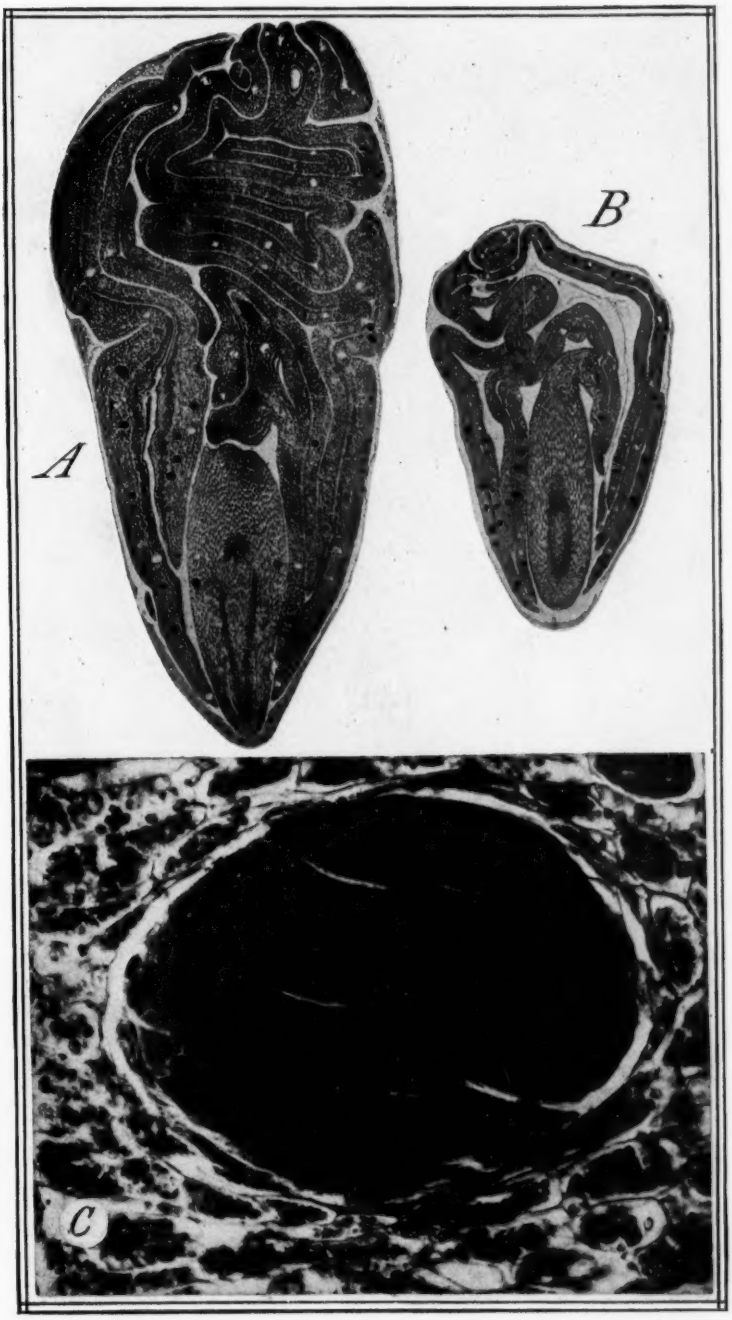
PLATE 42

A.—Longitudinal section of a cotton seed, showing the internal glands in the cotyledons and the radicle. × 12.

B.—Longitudinal section of seed of *Ingenhouzia triloba*, showing the internal glands as in *Gossypium* spp. × 12.

C.—Internal gland of a cotton seed, with secretion. × 450.


A
B
C

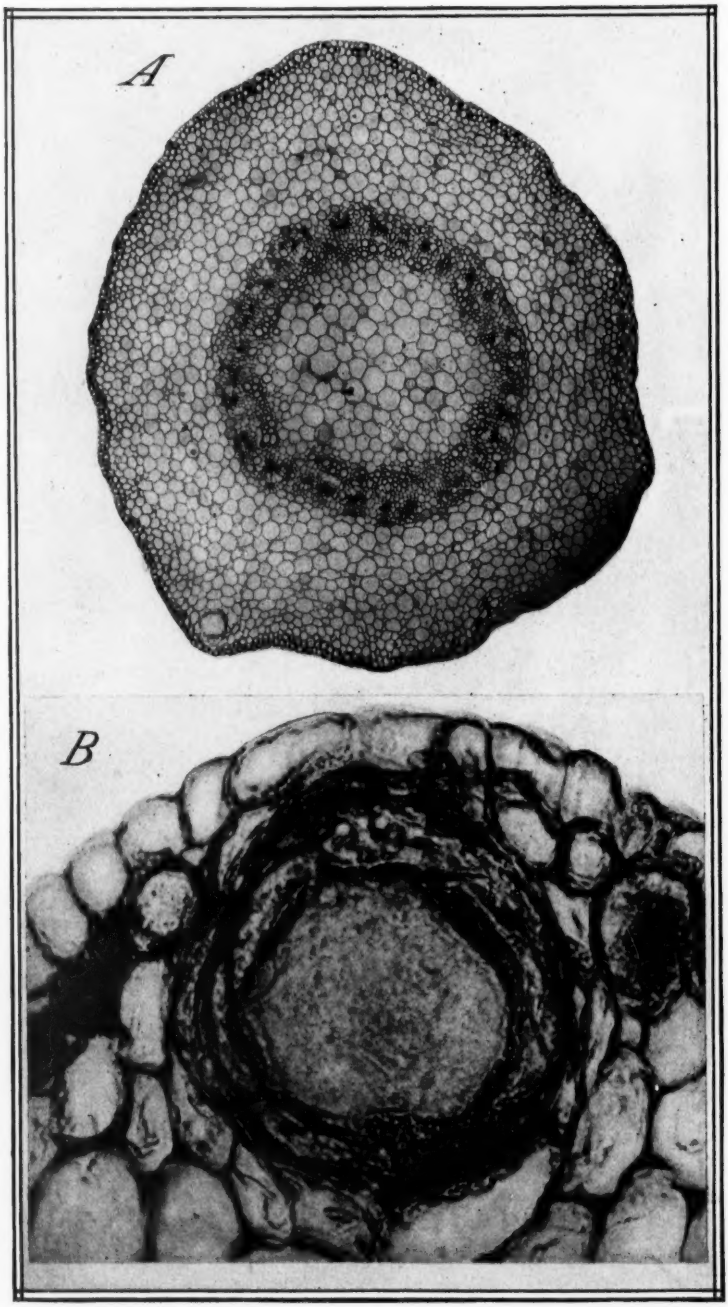

A
B

PLATE 43

A.—Cross-section of the hypocotyl of a cotton seedling, showing internal glands. × 48.

B.—Gland of same. × 655.

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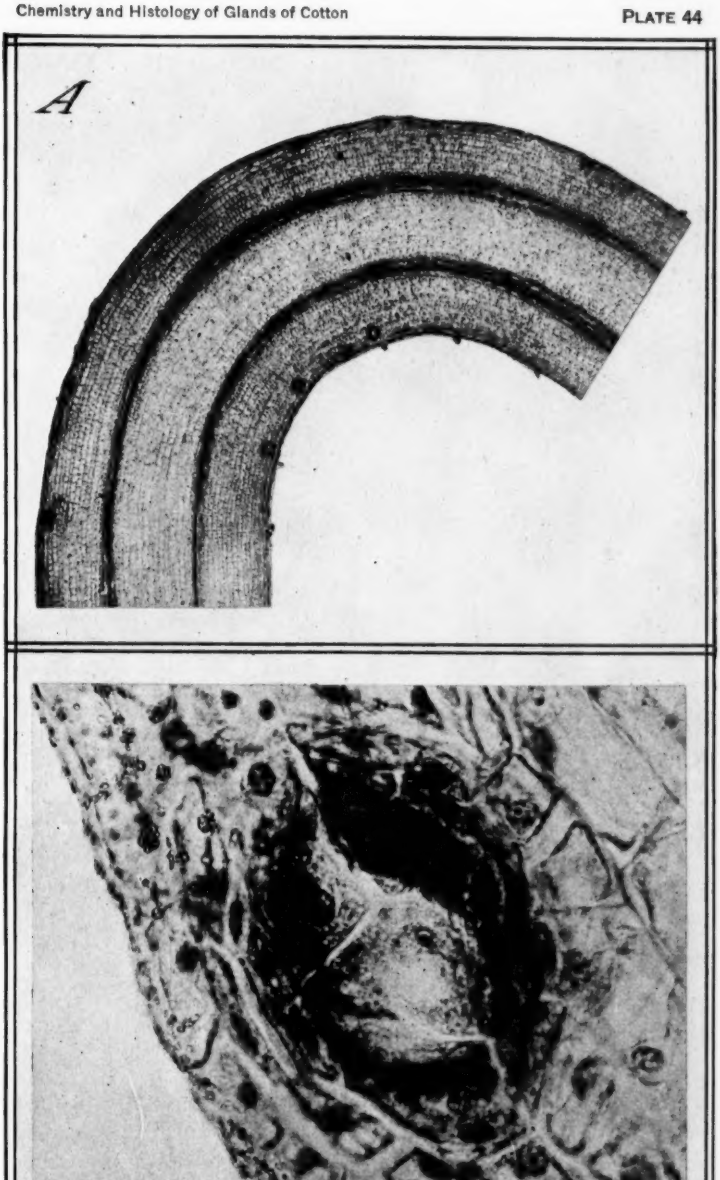
PLATE 44

A.—Longitudinal section of the hypocotyl of a cotton seedling, showing the internal glands. × 20.

B.—Gland of same. × 655.


A
B

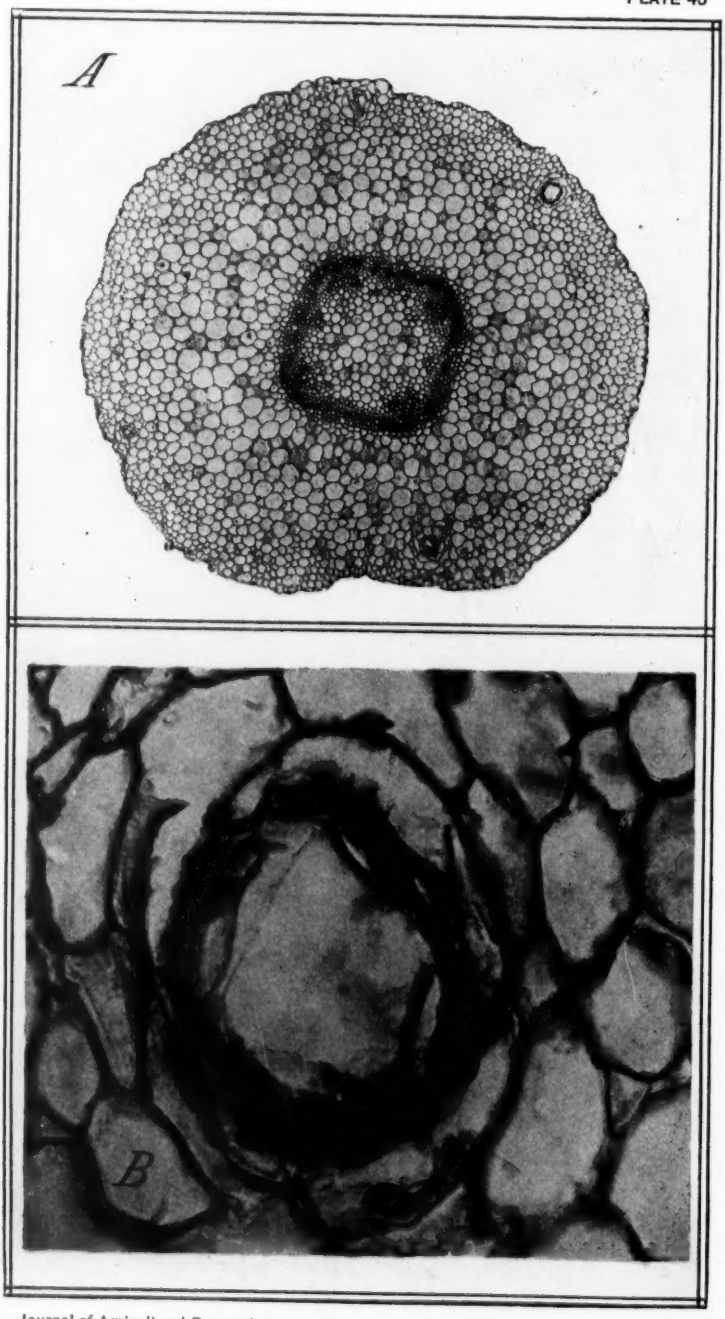

A
B

PLATE 45

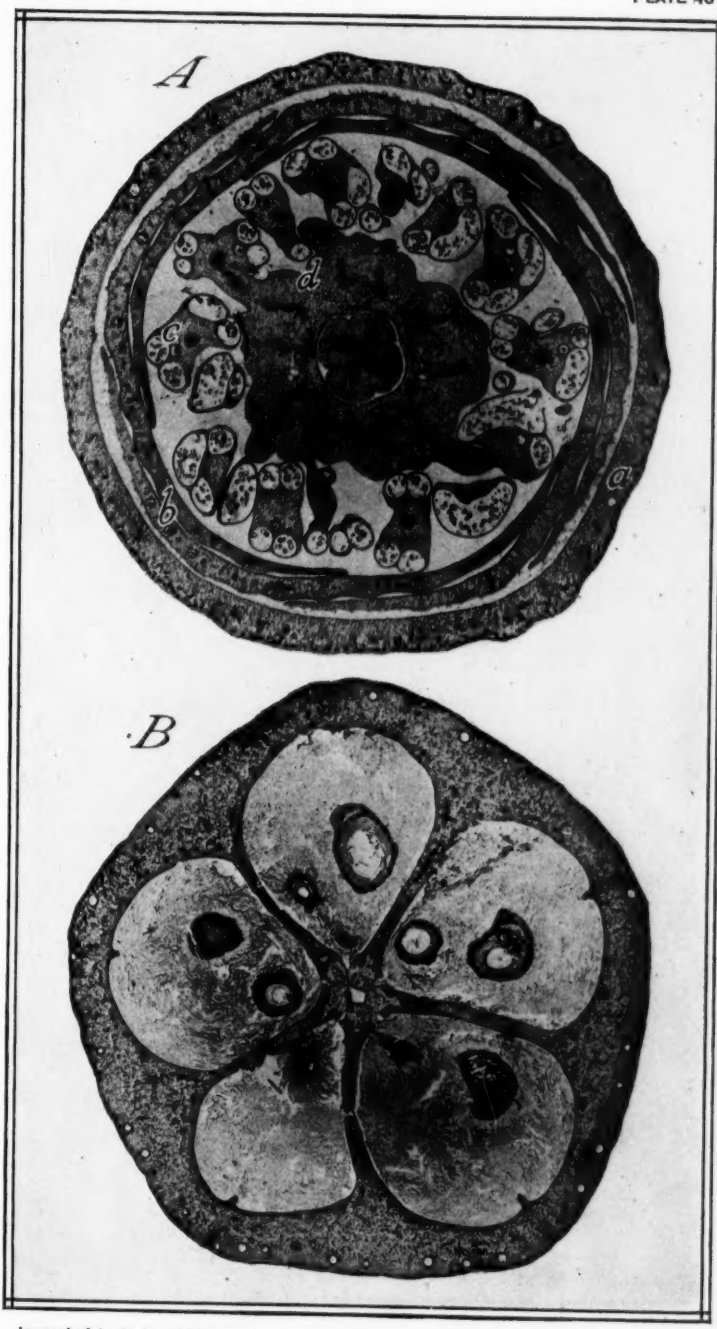
A.—Cross-section of a primary root of a cotton seedling, showing internal glands. × 38.

B.—Gland of same, the secretion having been removed by alcohol. × 585.

PLATE 46

A.—Cross-section of a cotton bud, showing internal glands in (*a*) calyx, (*b*) petal, (*c*) anther, (*d*) staminal column. × 15.

B.—Cross-section of a young cotton boll, showing internal glands. × 6.


A
d
c
b
a
B

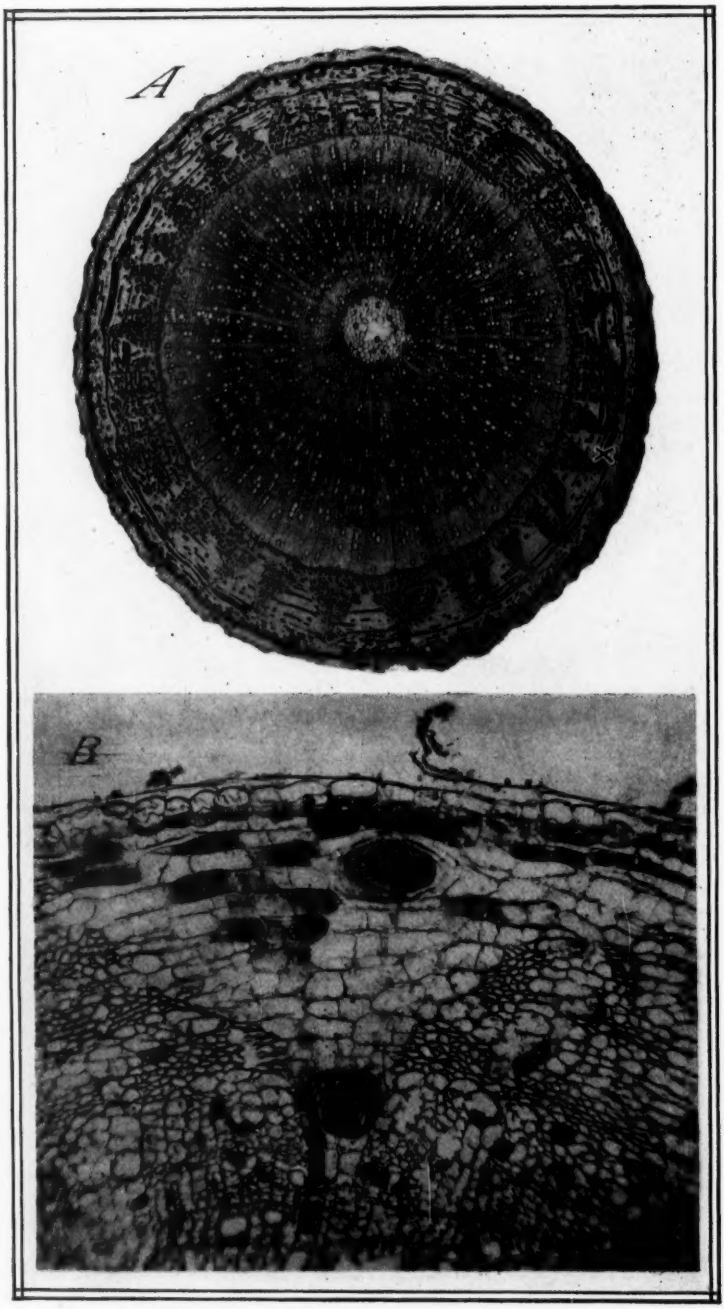

A
B

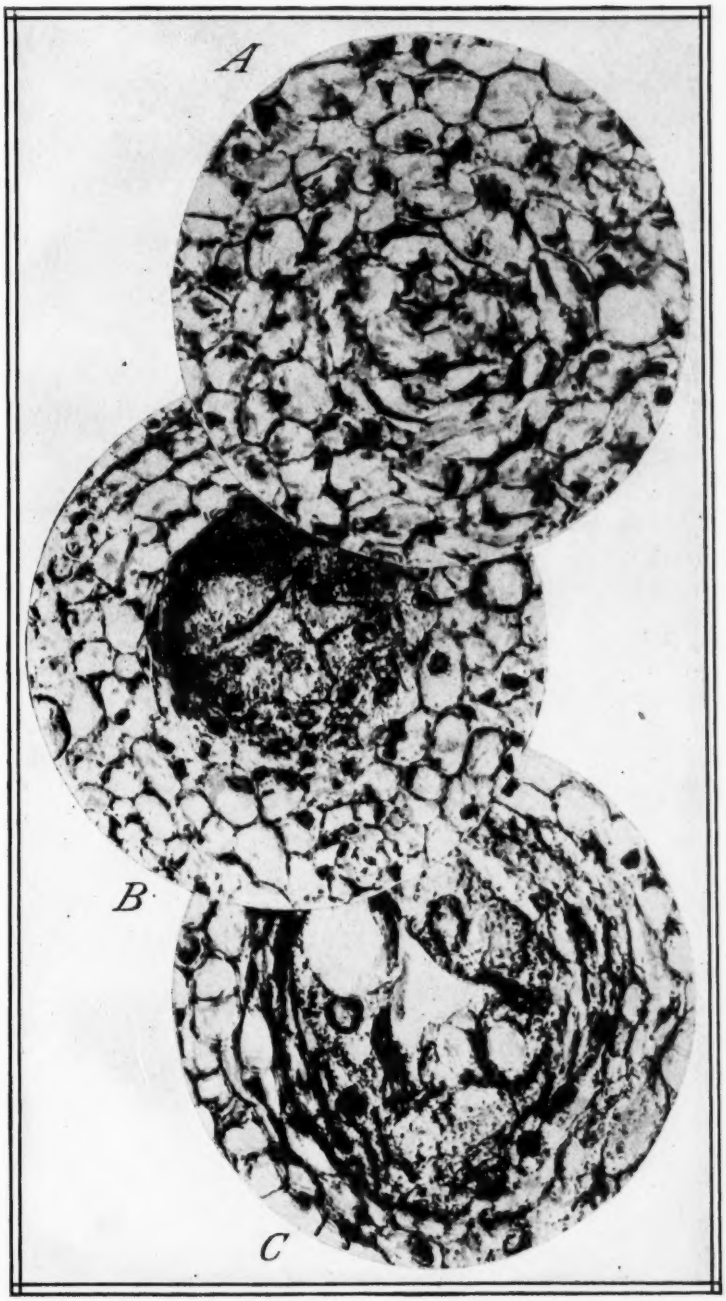
PLATE 47

A.—Cross-section of a woody cotton stem, showing internal glands in the primary cortex (X), but none in the secondary cortex. × 11½.

B.—Cross-section of a phloem ray of a cotton root, showing two internal glands × 98.

PLATE 48

A–C.—Cross-sections of the internal gland of cotton from the ovary in the bud, showing three stages of its development. × 655.


A
B
C

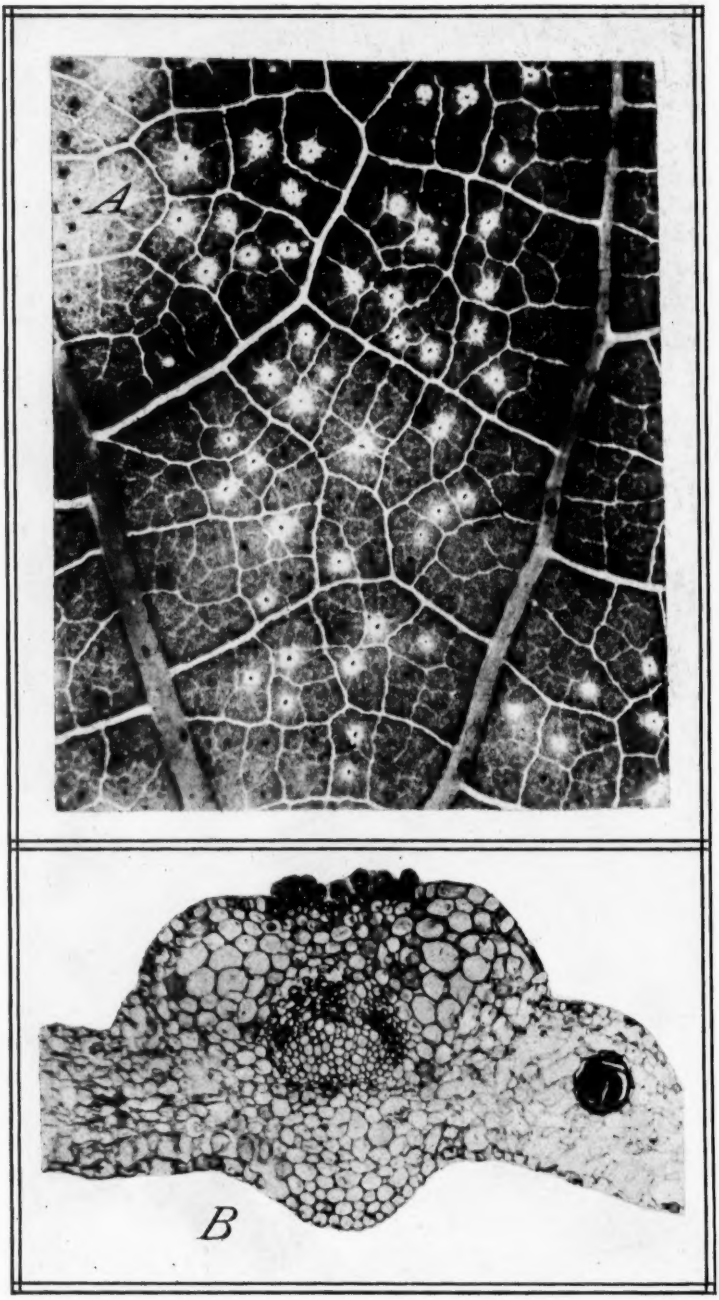

A
B

PLATE 49

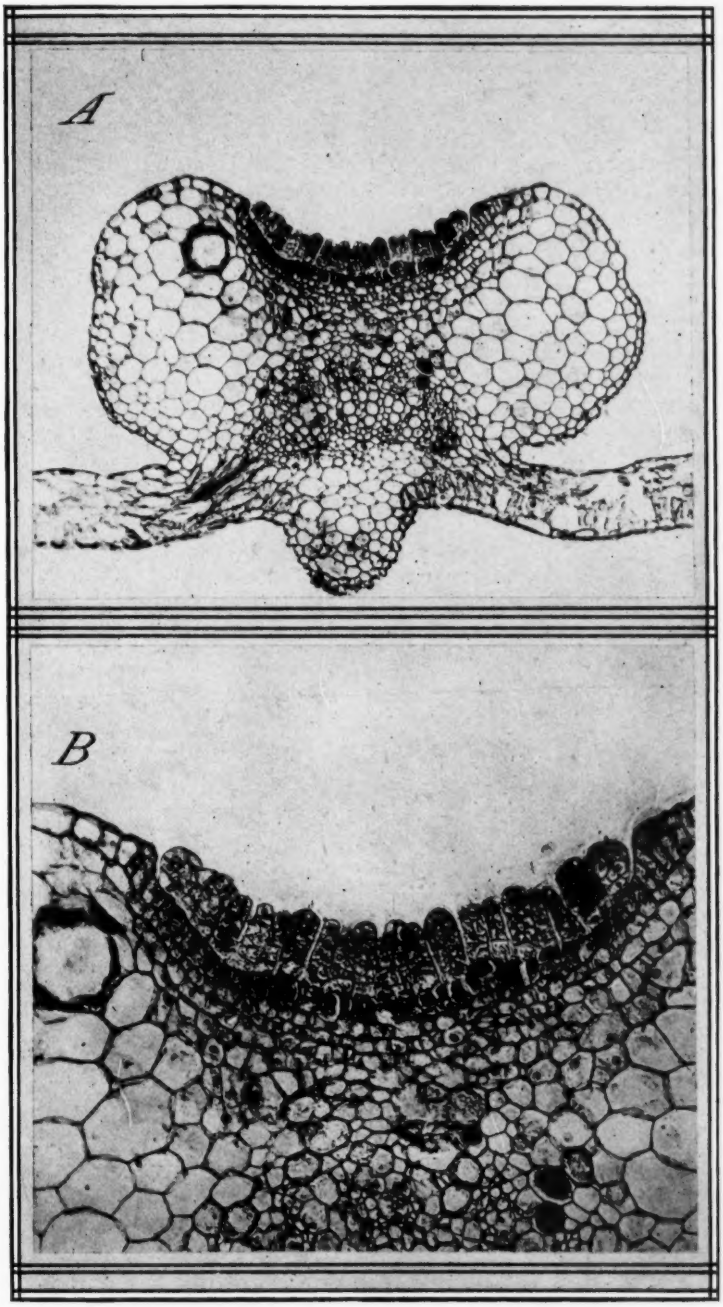
A.—Portion of a cotton leaf, showing internal glands punctured by aphids (surrounded by light area); also uninjured glands. Photographed by transmitted light. × 7.

B.—Cross-section of the midvein of a cotton cotyledon, showing rudimentary nectary. × 83.

PLATE 50

A.—Cross-section of the midvein of young true leaf of a cotton seedling, showing the nectary and internal gland. × 83.

B.—Nectary and internal gland of same. × 180.


A
B